STUDY ON CLINICAL PROFILE OF BETA THALASSEMIA MAJOR CHILDREN

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CERTIFICATE

This is to certify that this dissertation titled "**STUDY ON CLINICAL PROFILE OF BETA THALASSEMIA MAJOR CHILDREN"** submitted by **DR.M.S.NISHA** to the Tamilnadu DR. M.G.R medical university, Chennai in partial fulfilment of the requirement for the award of MD degree branch VII, is a bonafide research work carried out by her under direct supervision and guidance.

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DECLARATION

I, **DR.M.S.NISHA,** solemnly declare that the dissertation titled **- study on clinical profile of beta thalassemia major children** has been prepared by me. This is submitted to **The Tamilnadu Dr.M.G.R medical university, Chennai** in partial fulfilment of the regulations for the award of MD degree branch – VII Paediatrics.

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CONTENTS

INTRODUCTION

Beta-thalassemias are a group of hereditary blood disorders characterized by absence or reduction in the synthesis of the beta chains of hemoglobin. The resultant phenotypes varies grossly ranging from clinically asymptomatic individuals to those with severe anemia. Throughout the world the annual incidence estimated is around 1 in 100,000 for symptomatic beta thalassemics.

The average life expectancy of transfusion dependent beta thalassemics have increased to third and the fourth decades over the past ten years. Moreover the quality of these transfusion dependent children have been transformed due to better health care facilities. Nevertheless, the several complications of the disease have been disclosed as there is prolongation of life the complications may be partly due to the underlying disorder and is partly related to the conventional treatment with blood transfusions and subsequent iron overload. Moreover, in contest to the multiorgan disease, aging related complications have been emerging that adds up to the burden of the disease. These complications have to be care has to be dealt carefully for a proper management of the disease and its consequences. This masterly task demands dedicated work by a team of hematologists or clinicians who

have specific knowledge of thalassemias, different specialists and well trained nurses. The way in which the patients have been managed since childhood particularly with respect to their steady state hemoglobin levels and effectiveness of chelation therapy have major impact on outcome of the disease in relation to the frequency and severity of many complications.

In some of the developing countries individuals with thalassemia major are either untreated or transfused inadequately. Persistent severe anemia leads on to various consequences in these patients. The common findings in such underprivileged children include growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, and development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow.

On the other hand, complications due to iron overload contribute to the major issues in those patients receiving regular transfusion. The iron overload leads to serious complications including endocrine complication (growth retardation, , diabetes mellitus, and insufficiency of the parathyroid, thyroid, pituitary, failure of sexual maturation and less commonly, adrenal glands), dilated myocardiopathy, liver fibrosis and cirrhosis.

Long term packed cell transfusion therapy leads on to iron overload which is almost an inevitable and deadly complication. Iron overload can lead on to early death mainly from excess iron induced cardiac disease. This has to to be prevented by prompt and adequate treatment with removal of excess iron from the body i.e iron chelation. Quality of life of these patients as measured by the complication free survival can be extended by optimal chelation therapy, which has been proved beyond doubts in various studies throughout the world.the pst two decades have been filled with vast medical advnces in the treatment of thalassemia major including advances in transfusion regimes, better iron chelation measures ,and dramatic bone marrow transplantation. More than 200 mutations in β globin genes have been identified to cause the disease. The observed clinical heterogeneity in development of β thalassemia led to the identification of more than 200 mutations so far. The clinical spectrum displayed by the thalassemic individuals is dominantly influenced by the type of mutation inherited Delineation of the genetic repertoire for beta thalassemic mutation is pre-requisite for genetic counseling. These advances have definitely improved the prognosis for individuals with beta-thalassemia remarkably.

MATERIALS AND METHODS

Aim of the study :

- To study the clinical profile of β thalassemia major children on repeated packed cell transfusion – registered in pediatric hematology OP , Institute Of Child Health And Research Centre, Madurai.
- To identify the beta thalassemic traits among the family members of thalassemic children.
- To determine the distribution of beta thalassemic major mutations among thalassemic patients and family members attending Pediatric Hematology Unit, Govt Rajaji Hospital, Madurai.

Inclusion criteria:

- Known beta thalassemic children aged between 6 months to 12 years who are registered in pediatric hematology op , Institute Of Child Health And Research Centre, Govt. Rajaji Hospital, Madurai and on repeated blood transfusion were recruited for study

Exclusion criteria:

- Children with other hemoglobinopathies such as hemoglobin J variant etc were excluded from the study.

Methods:

- Children fulfilling the criteria are included in the study.
- Written consent obtained from the parents.
- Medical history taken with specific emphasis to family and treatment history
- Previous medical records were retrieved and analyzed
- Complete clinical examination was done
- Blood samples were collected for relevant investigations including blood grouping & typing, complete hemogram, iron studies, hemoglobin electrophoresis, blood sugar, bl.urea, s.creatinine, liver function tests, s.calcium, thyroid profile, viral markers for hepatitis, ELIZA for HIV.
- Oral glucose tolerance test, ultrasound thyroid were done when indicated.
- Radiological investigations including x rays of skull and chest, ultrasound abdomen, electrocardiogram, echocardiogram were done.

- All findings are recorded in well structured proforma.
- Molecular analysis of 42 individuals comprising of 16 patients (aged 6 months to 12 years) & 26 family members of 14 families were recruited for molecular analysis.
- Complete blood count and molecular analysis of mutation IVS1-5 G to C, Cd41/42 were performed as multiplex PCR at Dept of Immunology, Madurai Kamaraj University.
- Approximately, 3-5ml of peripheral blood (with EDTA, final concentration of 2.0 mg/ml) was collected, 0.5 ml of blood was utilized for performing complete blood count and remaining blood volume were transported to Dept of Immunology, Madurai Kamaraj University by maintaining the cold chain. Human genomic DNA was isolated from the blood samples by simple salting out procedure.
- Molecular Genotyping
- The β thalassemic mutation IVS1-5 G to C, Cd41/42 (-TCTT) were performed as multiplex PCR with internal control growth hormone. The allele specific mutation was performed individually with internal control primer and multiplex was optimized with minor modifications. The allele specific amplification was performed in 10µl reaction with 2µl of

25ng/µl DNA as template and final concentration of 1X Roche PCR buffer, 1.5 mM MgCl2, 0.4µM of allele specific primers, 0.04 µM of internal control primers, 0.4U/µl of *Taq* polymerase and 0.01% gelatine as PCR additive and amplification was performed in Agilent PCR Machine. 2.0% of agarose gel prepared with 1X TBE, 3µl of 1X Xylene cyanol FF added to 10 µl of PCR product. The allele specific amplicons IVS1-5 (285bp), Cd41/42 (500bp), internal control (1065bp) were electrophoresed for 25 mins at 100V. The amplicon was viewed after running the gel at 100 volts and it has been documented in Gel doc (BioRad Pvt Ltd).

Design of study: Prospective analytical study.

Period of Study: November 2010 – January 2012

Analysis: Statistical Analysis using SPSS package 17

Collaborating Departments: Departments of Pediatrics, Pathology, Biochemistry, Cardiology, Endocrinology, and Radiology, Government Rajaji Hospital, Madurai and Department of Immunology, Madurai Kamaraj University, Madurai .

Ethical Clearance: obtained

REVIEW OF LITERATURE

HISTORICAL BACKGROUND:

- β thalassemia is a monogenic hematological disorder first discovered by Thomas Cooley and Pear Lee in 1925 [1] (Cooley & Lee, 1925), later termed as Cooley'anemia or Mediterranean anemia.
- Whipple and Bradford [2], described the pathology of the condition and were first used to use the term 'thalassaemia' in their classical paper in 1932. The word thalassemia is derived from the Greek word 'qalassa', which means 'the sea'.
- The first description of clinical features of various types of thalassemias were published during the period between 1925 and 1940
- The clearer picture about the inheritance of the condition is obtained by by amalgamation of the information from Europe and united states from 1940 to 1960
- The pattern of genetic inheritance of thalassemia was first described during the period of 1940–1950
- Valentine and Neel[3] (1944, 1948) classifies cooley's anemia based on the severity of the disease naming the milder forms as thalassemia minor

and severe types s thalassemia major. They have done pioneer work on the genetic transmission of thalassaemia ,

- In 1946 Vecchio[4] noticed that the haemoglobin of Cooley's anemia patients was found to be more alkali-resistant than normal adult haemoglobin. Thereby he suggested that the amount of fetal hemoglobin is more in patients with cooley's anemia than what is usually present after first year of life.
- The microcytic anemia of cooley's disease is often accompanied by the presence of large, pale macrocytes and target cells . Dameshek [5] pointed out this phenomenon and called the condition 'target-cell anaemia' or 'leptocytosis'.
- In 1952 Rich [6] suggested that thalassemia results due a defect in HbA synthesis with persistent production of HbF .
- The important theoretical model for the genetic basis of thalassemia was set out by Ingram and Stretton[7] in 1952.
- The reasonably clearer picture of genetic control of thalassemia was obtained by 1960–1980.

- Kan and Nathan[8] in 1970 described a patient with mild form of β thalassaemia intermedia whose both parents both had raised levels of HbA2.
- The landmark in the treatment of thalassemia , Desferrioxamine , the iron cheltoe was introduced by Sephton-Smith[9,10] in (1962, 1964).
- The final unraveling of the molecular pathology of thalassemia was started in the year 1980 and thereafter.

DISTRIBUTION:

Most frequently, this disorder is found in the malarial, tropical and subtropical regions of Mediterranean countries, the Middle East, Central Asia, the Indian Subcontinent (South Asia) and Southeast Asia [11].

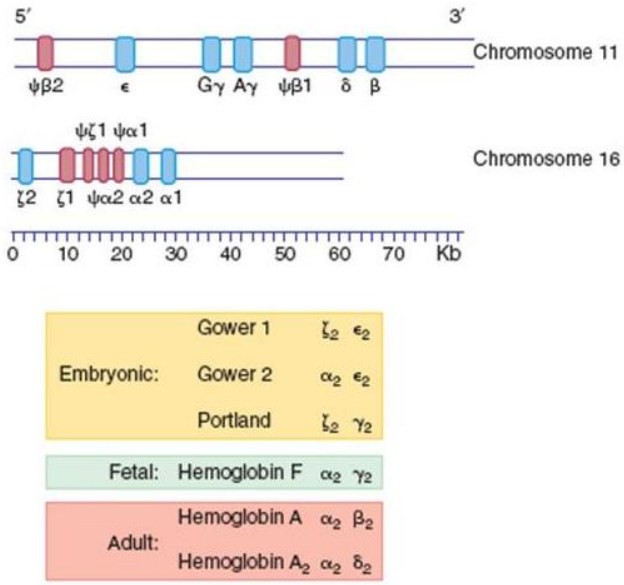
India is a vast and diverse country with a population of over a billion with considerable genetic diversity. Approximately 26 million infants are born in India each year which is almost 20% of the total world's infants. There have been several invasions from the Middle East, Central Asia and the West as well as commercial interactions through trade routes with people of Central, Western and South East Asia, the Mediterranean region and Western Europe over thousands of years of its history resulting in a remarkable racial and cultural mix as well as considerable genetic diversity.

The first case of thalassemia, described in a non-Mediterranean person, was from India. Subsequently, cases of thalassemia were documented in all parts of India. Every year 10,000 children with thalassemia major are born in India, which constitutes to about 10% of the total number born in the world each year.

Centers for care of thalassemia were started in the mid-1970s in Mumbai, Delhi and in other cities later. The International thalassemic federation and Indian Red Cross Society plays a crucial role in arranging voluntary donations of blood and helped in improving the care of thalassemics. More emphasis on thalassemia care is also taken by Government of India in the 12th 5-year Plan. Many states provide blood transfusion and chelation therapy free of cost. Further, bone marrow transplantation and cord blood stem cell storage facilities are available in a number of centres which improves the health care of these patients.

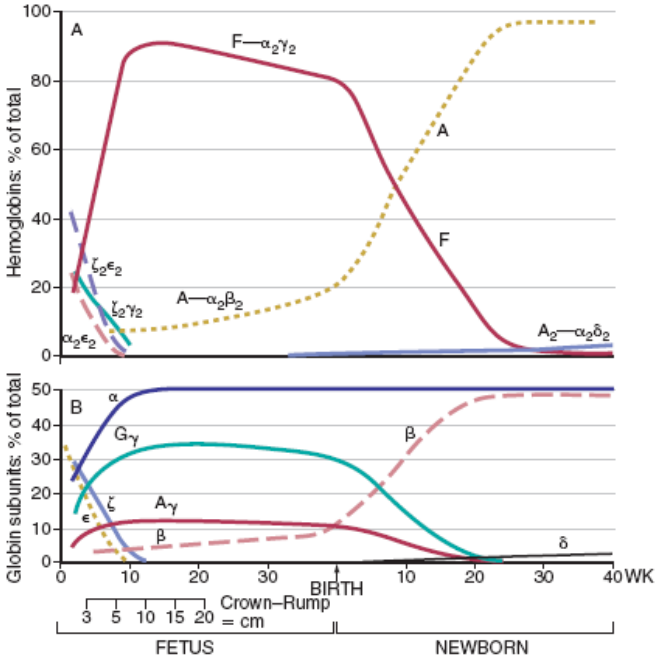
BIOLOGY OF β THALASSEMIA:

The β -globin gene is a small gene of 1.6 kb that occurs as a single copy in the haploid genome. It is arranged together with the other β -like globin genes (ε, Gγ, Aγ, and δ) in an ~60 kb long gene complex on the short arm of chromosome 11 (11p15) (Lin et al., 1985)[9]. Each gene is a separate transcriptional unit consisting of upstream regulatory sequences and the coding sequence (always split by two introns), followed by transcriptional termination signals that are probably important for RNA processing, mRNA stability, and efficient protein synthesis. Besides these local regulatory elements there is a region 6-18 kbp upstream from the β -globin gene that controls the expression of the entire β -globin gene complex during development and has been termed β –globin locus control region (β –LCR)



Normal adults have a major hemoglobin called HbA, comprising about 97% of the total, and a minor component,HbA2 which accounts for 2–3%. The main haemoglobin in fetal life is HbF, traces of which are found in normal adults. There are three embryonic haemoglobins, Hbs Gower 1 and 2. There is a series of physiological adaptations according to the differing oxygen requirements at various stages of development which is reflected by the production of these different hemoglobins. In addition, there are some minor haemoglobin components that are the result of postsynthetic modifications that may take place *in vivo*, or *in vitro* during storage in the laboratory. All the normal human haemoglobins are tetramers of two pairs of unlike globin chains. Adult and fetal haemoglobins have a chains associated with β(HbA, α 2β2), δ (HbA2,α2δ2) or γ chains (HbF, α2γ2), whereas in the embryo ζ chains combine

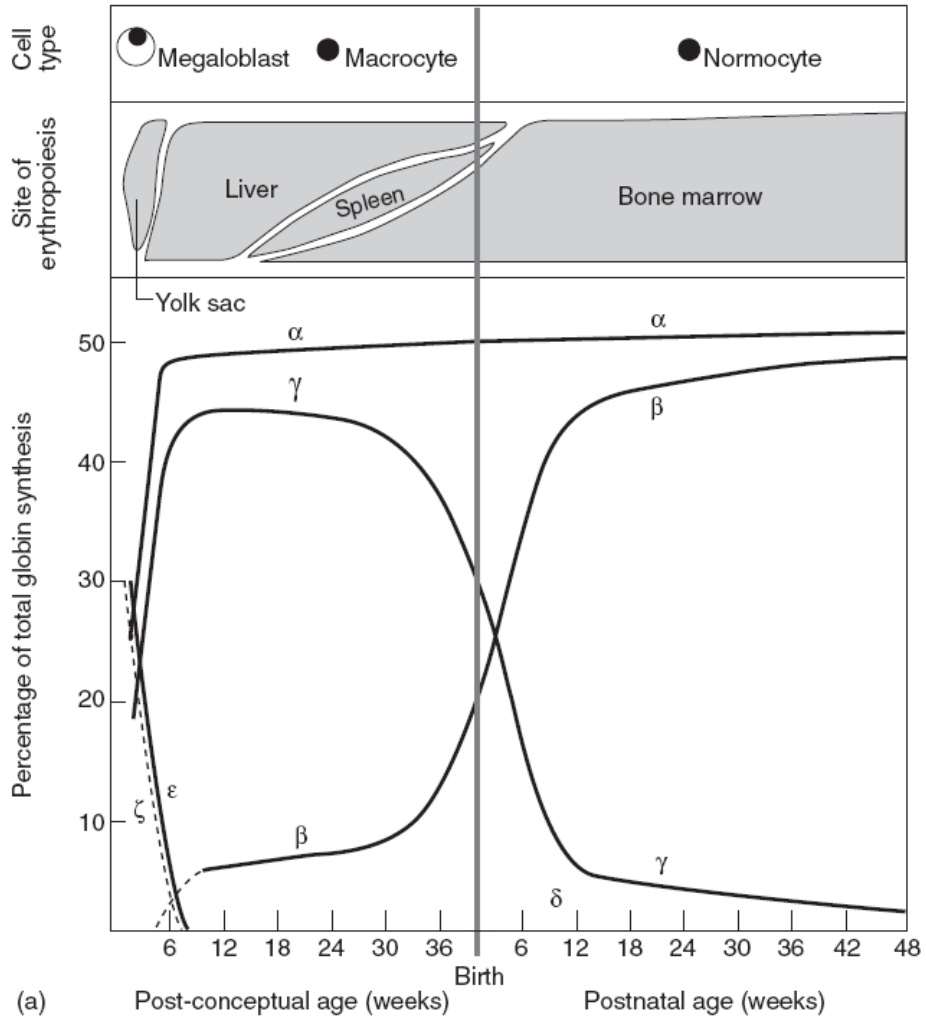
with γ (Hb Portland, ζ2,γ2) or ε chains (Hb Gower 1, ζ2,ε2), and α and ε chains combine to form Hb Gower 2 (α2ε2).



Developmental changes in hemoglobin production:

Human haemoglobin is heterogeneous at all stages of development, beginning with the youngest embryos and continuing throughout adult life. In embryos, haemoglobin synthesis is confined to the yolk sac, where Hbs Gower 1 (ζ2e 2), Gower 2 (α2ε2) and Portland (ζ2γ2) are produced. Synthesis of β chain becomes detectable at about 6 weeks. At around 7–8 weeks' gestation the liver becomes the major site of erythropoiesis, producing large enucleated red cells. Throughout most of fetal life HbF production predominates, with a small

amount (<10%) of HbA. At mid-term the bone marrow begins to take over as the major site of red-cell production, though erythropoiesis is also found in the spleen, as well as in other tissues. Towards the end of gestation there is a gradual and reciprocal switch from HbF to A production. At birth, the cord blood normally contains ~70% HbF and this declines to ~20% by 3 months, 7.5% at 6 months, and less than 2% by the age of 1 year. Both fetal and adult haemoglobins are produced in the same cell during the switching period, with a gradual increase in the proportion of cells containing predominantly HbA. The proportion of HbF continues to decline throughout childhood and probably throughout adult life.



The β thalassaemias

The basic defect in beta thalassemia is a reduction in the β globin chain production. On the other hand, α globin chins are produced in excess leading to imbalance in the production of globin synthesis. In all severe forms of beta thalassemia there is persistence of HbF synthesis beyond birth and infancy but in variable degrees. Yet overall HbF synthesis and output is essentially

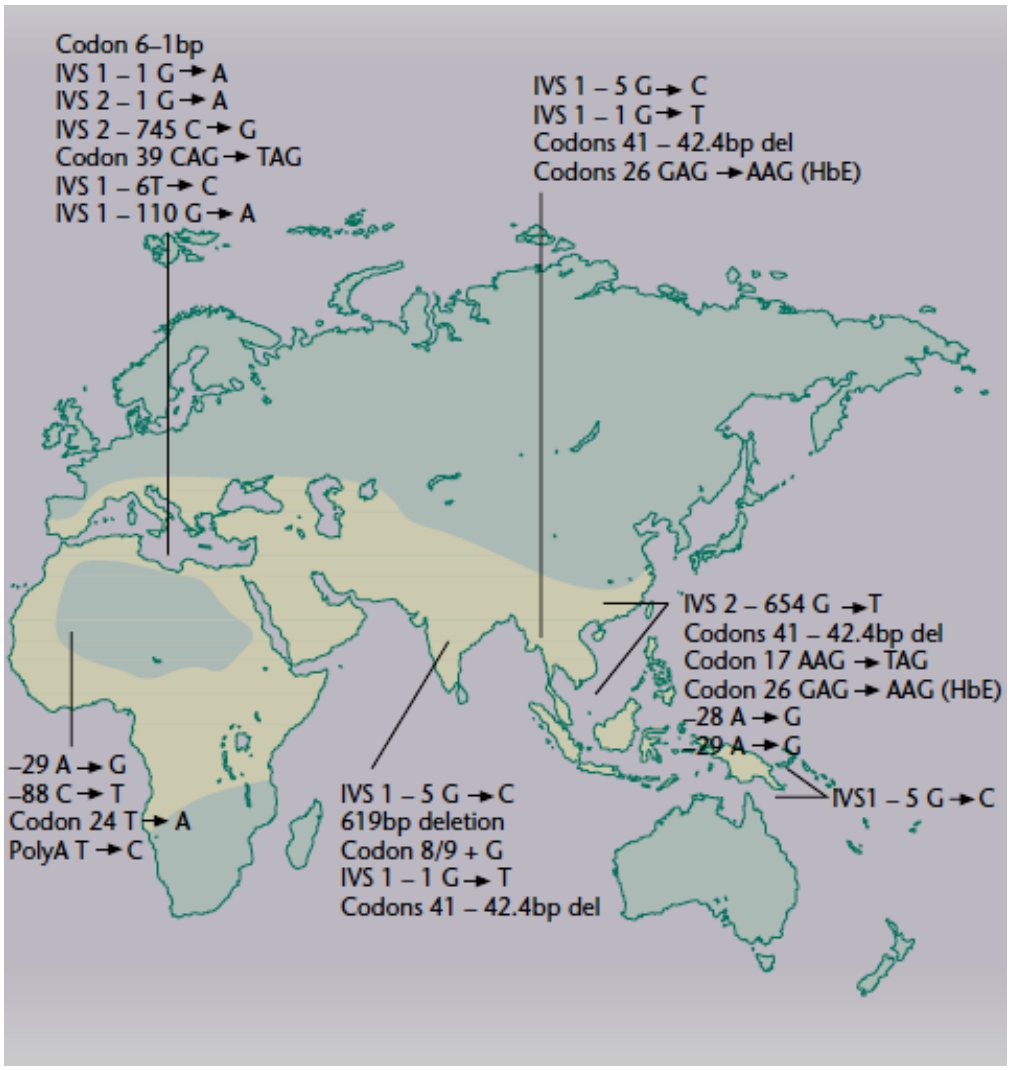
inadequate to compensate for the marked deficiency of HbA. In other words there is never a match between the production of α chains to that of output of β and γ chains chains in beta thalassemia. Thus the hallmark of this disease is the unbalanced production globin chains and an excess of α chains is therefore the pathogenic marker of β thalassaemia.

Classically in thalassemia, there is defect in the maturation of erythroid precursors along with ineffective erythropoiesis and a shortened red cell survival as the excessive unbound α chains precipitate within in the red-cell precursors in the marrow and in their progeny in the peripheral blood. There is intense proliferative drive in the ineffective bone marrow by the resultant anaemia , which leads to its expansion. Thereby a variety of growth and metabolic abnormalities develop and also results in n array of skeletal deformities. There is shunting of blood through the vastly expanded marrow spaces and the consequent hemodilution leads to further exacerbation of the anemia. This is augmented by the entrapment of the abnormal red cells in the enlarged spleen. The characteristic increased iron absorption and the consequent iron loading occurs due to the hyperplasia of the bone marrow which is often exaggerated by the need for regular packed cell transfusion. There is leads to progressive iron deposition in various tissues. If the excessive

iron that gets deposited is not removed by any means, multi organ failure ensues that culminates to death of these patients.

Mutations in beta thalassemia major

More than 200 mutations in β globin genes have been identified to cause the disease. The observed clinical heterogeneity in development of β thalassemia led to the identification of more than 200 mutations so far. Most of these mutations cause defects in transcription, RNA splicing, RNA modification and translation because of frame shifts and nonsense codons or produce highly unstable β -globin products. However, vast majority of β -thalassemia syndromes are caused by point mutations within the β –globin gene itself or in its immediate flanking sequences [37].

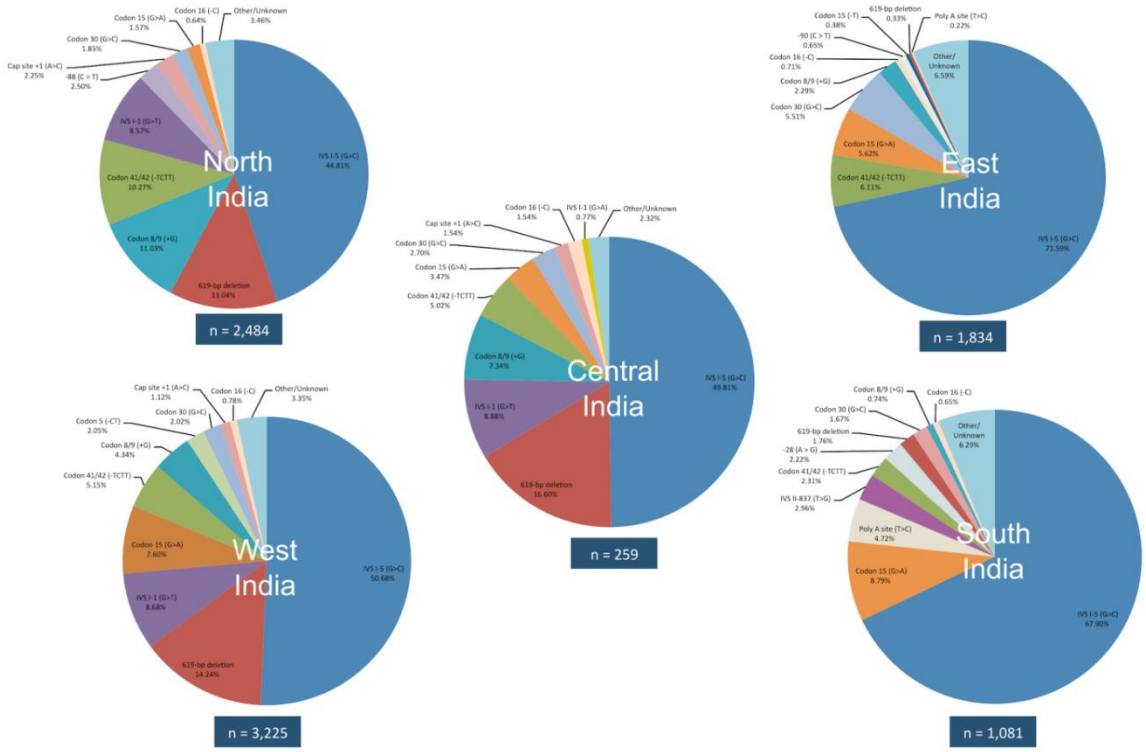

Codon 6–1bp
IVS 1 – 1 G → A
IVS 2 – 1 G → A
IVS 2 – 745 C → G
Codon 39 CAG → TAG
IVS 1 – 6T → C
IVS 1 – 110 G → A
IVS 1 – 5 G → C
IVS 1 – 1 G → T
Codons 41 – 42.4bp del
Codons 26 GAG → AAG (HbE)
IVS 2 – 654 G → T
Codons 41 – 42.4bp del
Codon 17 AAG → TAG
Codon 26 GAG → AAG (HbE)
–28 A → G
–29 A → G
IVS1 – 5 G → C
–29 A → G
–88 C → T
Codon 24 T → A
PolyA T → C
IVS 1 – 5 G → C
619bp deletion
Codon 8/9 + G
IVS 1 – 1 G → T
Codons 41 – 42.4bp del

Common types of beta thalassemic mutation : severity and ethnic types

Population	β-gene mutation	Severity
Indian	-619 del	β^0
Mediterranean	-101 CTT	β^{++}
Black	-88 CTT	β^{++}
Mediterranean; African	-87 CTG	β^{++}
Japanese	-31 ATG	β^{++}
African	-29 ATG	β^{++}
Southeast Asian	-28 ATC	β^{++}
Mediterranean; Asian Indian	IVS1-nt1 GTA	β^0
East Asian; Asian Indian	IVS1-nt5 GTC	β^0
Mediterranean	IVS1-nt6 TTC	$\beta^{+/++}$
Mediterranean	IVS1-nt110 GTA	β^+
Chinese	IVS2-nt654 CTT	β^+
Mediterranean	IVS2-nt745 CTG	β^+
Mediterranean	codon 39 CTT	β^0
Mediterranean	codon 5 -CT	β^0
Mediterranean; African-American	codon 6 -A	β^0
Southeast Asian	codon 41/42 -TTCT	β^0
African-American	AATAAA to AACAAA	β^{++}
Mediterranean	AATAAA to AATGAA	β^{++}
Mediterranean	codon 27 GTT Hb (Hb Knossos)	β^{++}
Southeast Asian	codon 79 G>A (Hb E)	β^{++}
Malaysia	Codon 19 G>A (Hb Malay)	

β^0:complete absence of beta globin on the affected allele
β^+:residual production of beta globin (around 10%)
β^{++}:very mild reduction in beta globin production

β-thalassaemia mutation distributions in five regions of India



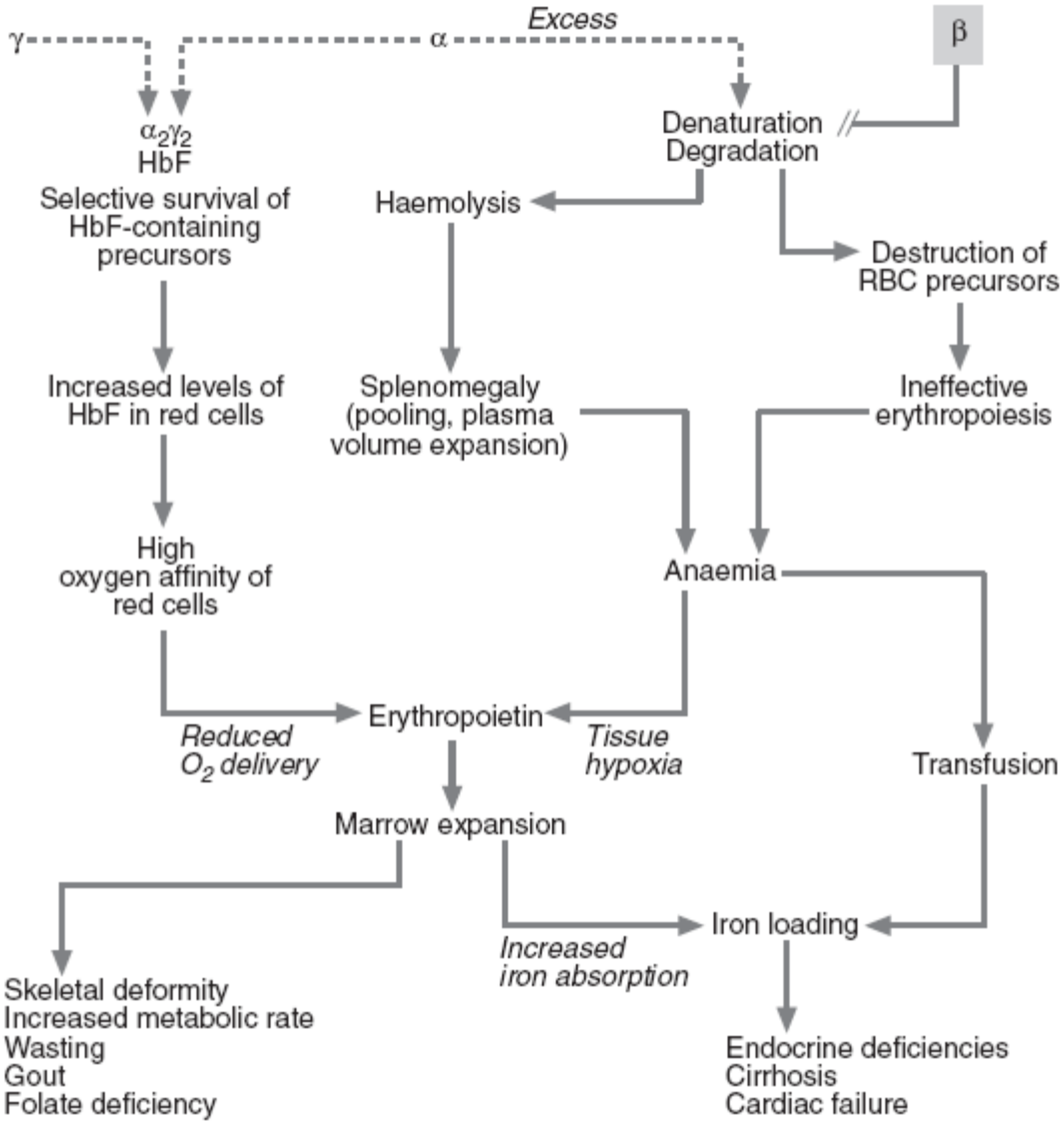
6 common Indian β-thalassemia mutations are [IVS1–5(G→C), CD 41/42(−CTTT), CD 15(G→A), etc. Distribution of these mutations varies among different regions. The commonest mutation in south India is [IVS1–5(G→C) amounting to 60%, and in Tamilnadu constituting to 56.5%.

The clinical spectrum displayed by the thalassemic individuals is dominantly influenced by the type of mutation inherited. Hence, diverse forms of thalassemia arise in mutations that affect nearly every step in globin chain expression i.e transcription, translation, processing and so on. Reduced or absent beta globin synthesis prevents adequate hemoglobin accumulation so that the cells are hypochromic and microcytic. Assessment of red cell parameters is the foremost laboratory investigation in the diagnostic workup of β thalassaemias. Routine diagnostic modalities for identification of β thalassaemia include low red cell values (MCV, MCH, RDW) on complete blood picture (CP), altered erythrocyte morphology and increased Hb A2 levels on high performance liquid chromatography (HPLC) or Hb electrophoresis. Mentzer index (mentzer et al.,1973)[10]calculated MCV/RBC count < 13.5 favors thalassemia over iron deficiency. In thalassemia, RBC production is preserved, so the RBC count is normal with a low MCV.

The clinical manifestations of the disease are due to a variety of pathological mechanisms.

Pathology	Consequence
primary mutation in genes coding globin chains	Imbalance in the synthesis of globin chains synthesis
Excess α chains that get precipitated in the red cell precursors and their progeny	Ineffective erythropoiesis and anemia
abnormal organ function	Anaemia, splenomegaly, hepatomegaly, hypercoagulable state
Severe anemia	Induction of erythropoietin production and marrow expansion with resultant skeletal deformity and metabolic abnormalities Adaptivity changes in cardiovascular function
abnormal iron metabolism	Iron overload which induces damage to liver, endocrine organs and myocardium
Therapy induced	Iron overload, , blood-borne infection, drug toxicity.

Outline summary of pathophysiology of beta thalassemia



Defective red-cell maturation and survival in β thalassaemia

Ineffective erythropoiesis:

The anemia in beta thalassemia is due to both ineffective erythropoiesis as well as shortened survival of the red cells. The ineffective erythropoiesis contributes the maximum to anemia as there is a large scale destruction of the erythroid precursors in the bone marrow. Ferrokinetic and erythrokinetic studies and concluded that the anemia is more predominantly due to ineffective erythropoiesis although the destruction of erythroblasts is also reflected in the pattern of bilirubin metabolism[12].

Haemolysis:

There reduced survival of abnormal red cells contribute to anemia in thalassemia patients but is found to less important than ineffective erythropoiesis in determining the severity of anemia. In various studies [13,14,15,17,18,19]Using either the Ashby or 51Cr-labelling methods, survival time of the red cells of thalassemics ranged from 7 to 22 days. Two other studies [13, 20] concluded that there are two populations of red cells, one which is very rapidly destroyed. There is evidence, that the longer-lived cells are richer in HbF while the shortlived population contains mainly HbA or α-chain precipitates. The

alterations in the membrane deformabilty , stability and the cellular dehydration of the red cells are probably because of the accumulation of the excess α chains at the membrane and its skeleton[21, 22]. The changes in the membrane characterized by a reduced spectrin/band 3 ratio, and partial oxidation and defective function of band 4.1 are associated with these abnormalities[23].

Secondary effects of ineffective erythropoiesis and anaemia:

Response to anaemia

The profound anemia induces increased production of erythropoietin in response to the chronic hypoxia. In pioneer study [24] found that there are significantly elevated Erythropoietin levels in the blood and as well as urine of patients with haemoglobin values of 7.0 g/dl or less.

Erythroid expansion

In beta thalassemia there is ineffective erythropoiesis and associated expansion of the ineffective erythroid mass that is estimated to range between 10 and 30 times normal in some cases [25, 26] . This uncontrolled expansion of the erythroid mass is of profound importance in the generation of most distressing clinical features of the disease, particularly bone deformities and, occasionally,

the production of extramedullary tumour masses. In young children, it also imposes an excessive metabolic burden. As a consequence these children fail to grow, poor muscular development, and reduced body fat and weight . There is exacerbation of the anemia undoubtedly due to shunting of blood through the massively expanded marrow together with splenomegaly[27] . There is high output state in profoundly anemic thalassemic children due to anemia and associated hypervolaemia that combine to produce cardiomegaly. There is elevated level of urates in urine and serum uric acid levels when compared to control subjects is evident as there is increased destruction of red-cell precursors.

Splenomegaly and hypersplenism

Mechanisms

One of the functions of the spleen is to act as a filter, retaining defective blood cells and foreign particles in a bed of phagocytes[28] . Approximately 5–10% of splenic blood is diverted into the red pulp and slowly percolates through a non-endothelialized mesh containing macrophages, after which it reenters the circulation through narrow slits, measuring 1–3 μm, in the endothelial sinuses. Sometimes called ‘work hypertrophy’, a term which at least hides our total ignorance of the mechanisms involved,the exposure of the

reticuloendothelial elements of the spleen to abnormal red cells like those of β thalassaemics leads to its progressive enlargement. This concept is supported by the observation that children who have received regular blood transfusions from early in life, and hence who do not have many abnormal red cells in their circulation, do not develop significant splenomegaly. The early observations in 1963 showed that blood cells carrying inclusions only appear in the peripheral blood after splenectomy also pointed to the central importance of the spleen in the pathophysiology of the anaemia[29]. Extramedullary haemopoiesis may also contribute to splenomegaly, and hepatomegaly.

Consequence of splenomegaly

Splenomegly leads to entrapment of all the formed elements of the blood , producing anaemia, thrombocytopenia and neutropenia. The anaemia in beta thalassemia has a complicated basis, which includes ineffective erythropoiesis with shortening of the red-cell survival, a dilutional element caused by pooling of a proportion of the red-cell mass in the spleen, and the ill-understood effect of increasing the plasma volume.

In the study 9 to 40% of the total red-cell mass were found to be entrapped in the splenic pool[18] . In severe forms of beta thalassemia spleen is also the site of extensive extramedullary haematopoiesis .Interestingly, Blendis

[18] *et al*. (1974) noted that splenectomy may be associated with a growth spurt, suggesting that an enlarged spleen might have a deleterious effect on development.

Plasma volume expansion

Plasma volume expansion worsens the anemia and also poses a greater load on the myocardium. The plasma volume doesnot return normal after splenectomy suggesting that the plasma volume expansion is not entirely due to splenomegaly[18]. It has been suggested that vascular shunt mechanism across the vastly expanded bone marrow results in plasma volume expansion, As in other diseases which result in expansion of plasma volume.

Iron overload

One of the most well recognized and age old complication of thalassemia is generalized iron overload and deposition iron in tissues and organs[2,16,30,31,32] Both the erratic absorption of iron from the gut as well as from transfusion delivers excess iron into the body system. The patients who are either inadequately transfused and those with intermediate forms of thalassemia, increased absorption from the gut is considered to be the predominant mechanism of iron over load. On the other hand, in patients who

are adequately transfused children the latter mechanism predominates as the major route of iron overload.

Mechanisms and rate of iron loading

The amount of iron in the body stores and the level of erythropoietic activity are the two major factors that influence the rate of absorption of iron from the intestine. A unit of blood contains approximately 200 mg of iron and, since there is no natural way by which iron can be excreted from the body, a regular transfusion regimen rapidly increases the body iron stores. Additionally iron absorption from the gut increases dramatically in the presence of ineffective erythropoiesis and erythroid expansion; the drive to increased iron absorption overcomes the physiological mechanisms whereby it is normally reduced in the presence of increased body stores.

Although being life saving in these patients, red cell transfusions are responsible for series of complications and thereby exposes the patient to a variety of risks. Among all the complications of regular red cell transfusion therapy iron overload is the most relevant complication.

Mechanisms of tissue damage in iron loading

Although iron is vital for living processes, excess iron can generate extremely toxic free radicals, which cause widespread tissue damage under certain conditions. Normally iron is tightly bound to storage or transport proteins; for example catalytic effect of iron in free radical production can be prevented by binding of plasma iron to transferrin. [33]. However,when the transferring gets saturated with the increasing levels of iron overload, non transferrin bound plasma iron (NTPI) becomes detectble in the blood which is potentially toxic to body[34,35,36]. Moreover , the low molecular mass iron that is present in the serum of patients with iron overload is also present in many other tissues[32]. Generally iron is usually tightly associated with haem and non-haem proteins such as ferritin, transferrin and haemoglobin, but on imposing an oxidant stress on iron-containing proteins can release some 'free' iron. The most important pathological consequences of iron overload result from involvement of the liver, heart and endocrine glands despite the fact that iron gets deposited

Clinical consequences of iron overload:

Iron overload is attributes to most of the complications of the disease.heart, liver and various endocrine glands are the most commonly

affected organs. The commonly affected endocrine glands include pituitary gland, thyroid gland, parathyroid gland, pancreas, gonads. Clinically they may not be evident initially and hence investigations are required for early detection and should be done in all thalassemia children from time to time and treat them appropriately. Diabetes may be seen as early as five years of age. Dysfunction of thyroid and parathyroid gland may be subclinical initially, so blood sugar estimation, thyroid function assessment, s. calcium should be done frequently. Liver is affected by due to various causes including repeated blood borne infections and excessive iron deposition. Hence it is essential to do liver function tests and viral markers frequently atleast once in 6 months.it is also important to monitor the organ functions regularly particularly such as heart, endocrine glands, growth failure and complications due repeated infections. Growth failure is seen in nearly 30 % of western children and and nearly all children in our country. The mean age of attainment of sexual maturity is also delayed. Various cause have been attributed to growth retardation include poor compliance to regular blood transfusion, inadequate chelation, growth hormone deficiency secondary to pituitary hemosiderosis, defective hepatic biosynthesis of of somatomedins and sex hormone deficiency and chronic hypoxia secondary to anemia. Treatment of subclinical hypothyroidism is debatable. Close monitoring of the patients is necessary when treatment is considered as

unnecessary. In overt hypothyroidism characterized by low T4 levels with signs and symptoms such as mental and physical letharginess, cold intolerance, weight gain, constipation etc, treatment with L- thyroxine is considered. Abnormal thyroid function may be reversible t the early stage through intensive chelation therapy.

Cardiac complications leads to 70% of deaths in beta thalassemia, which include cardiac failure and arrhymias. Excess iron gets deposited in the heart especially in ventricular walls and the conduction system. When iron accumulates in the cardiac tissue, free iron damages the cells sue to lipid peroxidation and lysosomal rupture. Cardiac complication in thalassemic children include overt cardiomyopathy, dilatation of the left atrium, dilatation of the aortic root dilatation , reduction in the internal dimension of left ventricle both in systole and diastole. Early detection of cardiac involvement can be done by evaluation of ferritin levels and various tests to evaluate cardiac functions like ECG, echo etc. All these tests can only assist in evaluation of cardiac involvement , but donot quantitate cardiac evaluation. The best available method to assess the severity of cardiac evaluation is T2 weighted cardiac MRI but it is available only in certain centres now.

Management of thalassemia major

- Correction of anemia by packed cell transfusions
- Removal of excess iron by chelation therapy
- Management of complications
- Curative treatment : stem cell transplantation
- Future treatment: gene therapy
- Prevention of the disease by genetic counseling, prenatal diagnosis and preimplantation genetics

Transfusion therapy

The goals of treatment with transfusion is to correct the anemia and to suppress ineffective erythropoiesis. Regular packed cell transfusion is presently the mainstay of treatment.

Type of transfusion	Pre transfusion Hb	Mean Hb maintaineed
Palliative	<7g%	<8.5 g%
Hyper transfusion	>10g%	>12 g%
Super transfusion	>12 g%	>14 g%
Moderate transfusion	9 -10.5 g%	>12g%

Current recommendation is to maintain the mean post transfusion Hemoglobin levels of 12g% and transfuse the child at the pretransfusion level of 9 to 10.5 g%. (moderate transfusion). Post transfusion hemoglobin should not rise above 15 -16 g%.

Transfusion dependent complications	
Iron overload	
Infections	- Viral (HIV, HCV, HBV, HTLV1, west nile virus - Bacterial - Parasitic - Creutzfeld – Jacob disease - Emerging and new pathogens
Haemolytic reactions	-acute haemolytic rections - delayed haemolytic reactions. - autoimmune haemolytic anemia
Non haemolytic reactions	- Allergic and anphylactic rections - Febrile non haemolytic reactions - Transfusion related acute lung injury - Transfusion associated graft versus host disease - Circulatory overload - Post transfusion purpura

Chelation therapy:

Iron overload is the main problem encountered in the management of thalassemia. . As there are no effective mechanisms for excretion of iron from the body , the use of iron chelators is the the only way for the removal of excess iron . The use of iron chelators is mainly aimed at reducing the iron stores in the body and to maintain the iron store in the body at low levels.The drugs used presently include desferroxamine , Deferiprone, deferasirox.

Desferrioxamine : The dose is 30 -40mg/kg/day givesubcutanuoesly over 8-10 hours for 6 nights a week using subcutaneous desferal infusion pump. Depot desferrioxamine is a newer modification of chelation therapy.

Deferiprone : The dose is 75 -100mg/kg/day in three to four divided doses orally. It found to be 70 -100% as effective as desferrioxamine and leads to effective reduction in both serum ferritin and tissue iron overload.

Deferasirox : Newer oral iron chelator for treatment of iron overload associated with chronic blood transfusion. The dose is 20 -40mg once daily adjusted upon patient's response, serum ferritin and serum creatinine levels. It is found to be nearly five times as effective as subcutaneous desferrioxamine and ten times more potent than deferiprone in animal studies

As a general rule, chelation therapy should be started patients with thalassemia major once they had received ten to twenty transfusions or when serum ferritin levels rises above 1000µg/dl[55].

Prospective and retrospective studies have shown that myocardial sidereosis is reduced more effectively by deferiprone monotherapy and has reduced the cardiac morbidity and mortality[48,49,50].

Intensive therapy with iron chelators seems to bring about improvement in glucose tolerance, abnormal thyroid function and other ill effects of iron overload in early stages.[51,52,53].

Future perspectives:

Newer drugs like PIH (pyridoxal isonicotynoyl hydrozone) HBED- and dimethyl HBED though looked promising as they are relatively non toxic and effective, however they are non patentable.

Pharmacological manipulation of HbF inducing drugs like hydroxyl urea, butyrates, 5-azacytidine[54]. Pharmacological gene manipulations heve been tried in order to increase the production of HbF and to prevent the precipitation of unpaired Hb chains.

Indications for splenectomy:

- When the yearly requirement of packed cell transfusion increases more than double the basal requirement. i.e. around 230 -250 cc per Kg.
- Decreased platelet count – relatively late manifestation of hypersplenism.

All patients needing splenectomy should receive Pneumococcal vaccine , H. influenza type b vaccine and meningococcal vaccine about 6 to 8 weeks prior to splenectomy . Splenectomy is better avoided in children less than 5 years of age.

Stem cell transplantation: is the only curative treatment available at present with a ray of hope for permanent cure and better future for children with genetic disorders such as thalassemia.

Prenatal diagnosis and genetic counseling:

Prevention of beta thalassemia major is generally based on carrier detection, genetic counseling and prenatal diagnosis. Genetic counseling is usually given for individuals and at risk couples (i.e. both carriers). Information regarding the mode of inheritance , the genetic risk of having

affected children and the natural history of the disease including the available treatment and therapies under investigation are provided.

Prenatal diagnosis is possible by the analysis of DNA extracted from the fetal cells obtained by amniocentesis or chorionic villi sampling for pregnancies at increased risk. Identification of disease causing mutation is a must be before prenatal testing is performed. Currently analysis of fetal cells in the maternal blood and analysis of fetal DNA in maternal plasma for the presence of father's mutation are under investigation. Preimplantation genetic diagnosis can be offered to families in which the disease causing mutations have been identified.

Pre implantation genetics

A newer genuine method of prevention of disease would be pre implantation diagnosis by the polymerase chain reaction (PCR). In this method the isolation of 1-2 blastomeres from embryos or, alternatively, aspiration of a polar body from oocytes is performed .Ideally if the mutation causing the disease is excluded , the remaining blastomeres are transferred into the mother's womb for normal fetal development [38]. In future there is a expected possibility to provide a comprehensive genetic screening of embryos fertilized and developed in vitro and thereby reduction or elimination of beta

thalassemia by the micromanipulation of the gamete and embryo biopsy combined with the sensitive PCR technology [39].

Prevention of beta thalassemia

Only 10 to 15% thalassemic children born in India receive optimal treatment. The cost of treatment for thalassemic child is around Rs.1,00,000 annually. Curative treatment in the form of bone marrow is not affordable by most of our patients. The birth of thalassemic child places considerable physical, economic and emotional strain not only to the child and the family but also to the nation. So the emphasis must shift from treatment to prevention of such births. Prospective prevention which includes population education, mass screening, genetic counseling, and prenatal diagnosis and possibly preimplantation genetics, is the only effective way to cope successfully with such a disease. Various screening tests have been used to perform mass screening in population. These include menzter's index, MCV, NESTROFT (Naked Eye Single Tube Red cell Osmotic fragility test) etc. However, none can estimate the confirmatory HbA2 estimation for the definitive identification of beta thalassemia carriers. Those who are confirmed to have thalassemia trait should be counseled for testing their partner. If both are tested to be positive, they need to be counseled regarding prenatal diagnosis in first trimester with

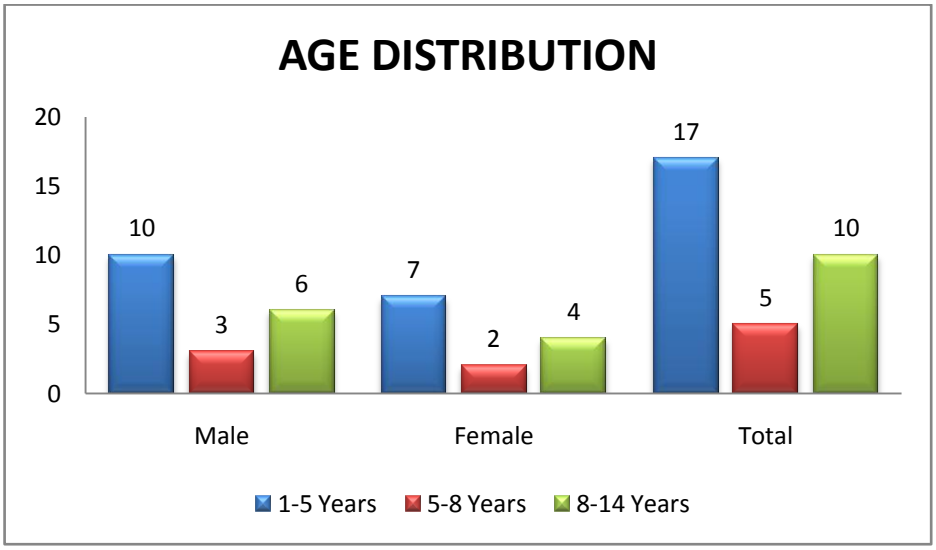
chorionic villi sampling and in the second trimester with amniocentesis. Thus every baby born to two carriers of thalassemia should be screened in utero and if those fetuses affected , termination should be advised.

OBSERVATION AND ANALYSIS

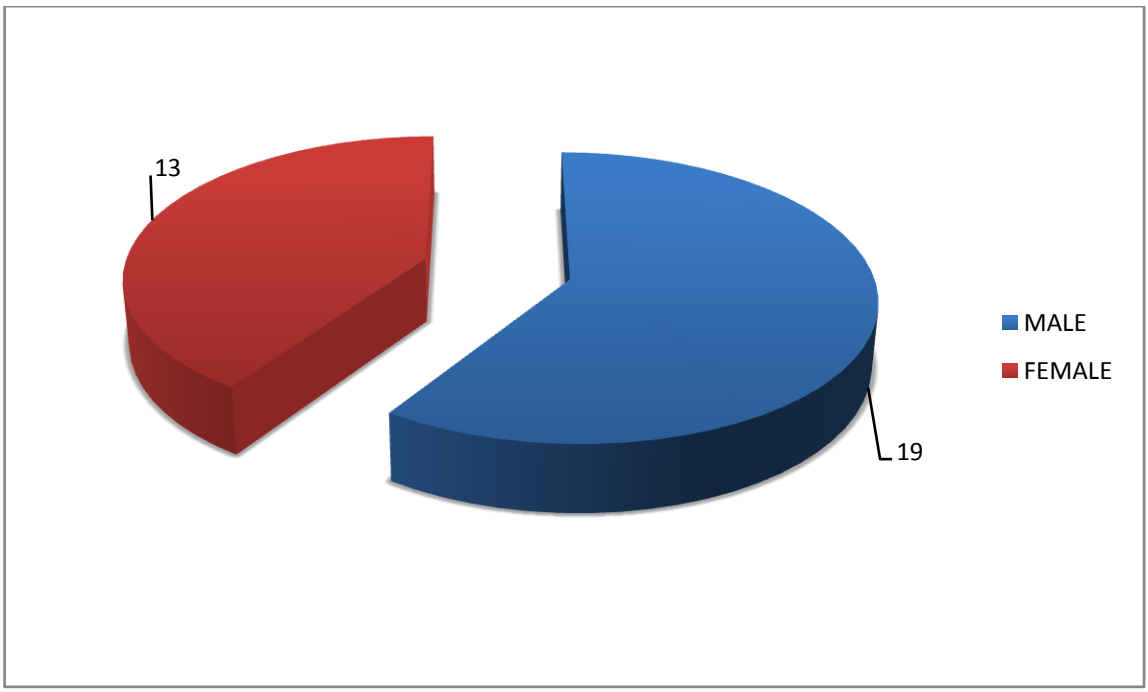
Total number of children included in the study: 32

Sex and age distribution:

AGE DISTRIBUTION			
Years	Male	Female	Total
1-5 Years	10	7	17
5-8 Years	3	2	5
8-14 Years	6	4	10

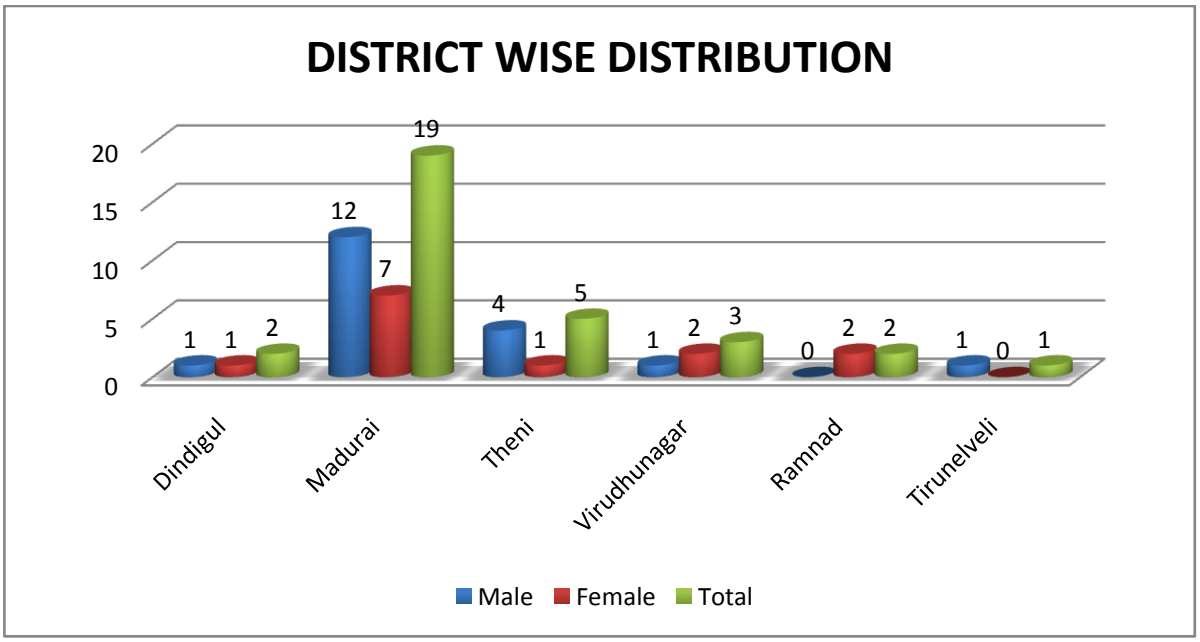


Sex	No of patients
MALE	19
FEMALE	13



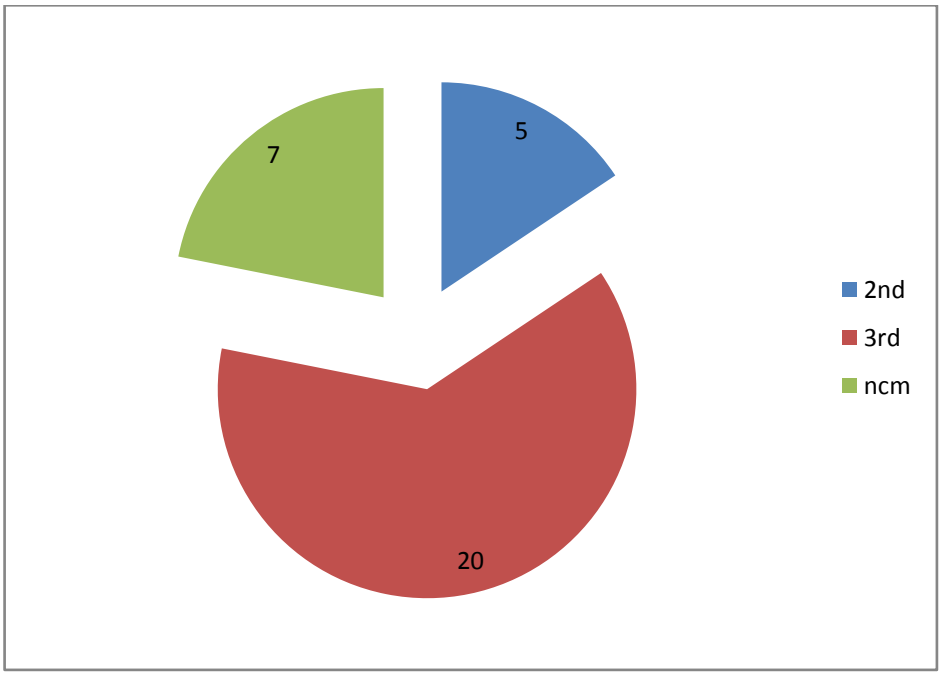
District wise distribution

	Male	Female	Total
Dindigul	1	1	2
Madurai	12	7	19
Theni	4	1	5
Virudhunagar	1	2	3
Ramnad	0	2	2
Tirunelveli	1	0	1



Consanguinity

Degree of consanguinity	No of families
2nd	5
3rd	20
ncm	7



Family history:

- None of the parents were affected
- 3 pairs of siblings affected.

Transfusion details

- All children were repeated packed cell transfusions
- Average pre transfusion Hb -5 -7 g%
- Children received around 20 transfusions per year.
- Average interval between the transfusion – 3 to 4 weeks

Chelation details:

- All beta thalassemic children were on oral iron chelation therapy
- Irregular compliance to the drug.

Splenectomy :

- 2 out of the 32 cases had undergone splenectomy

Blood sugar:

- Blood sugar elevated in 2 children out of 32 children
- On glucose tolerance test – impaired glucose tolerance
- Mean value – 99.75 mgs% & Standard deviation – 41.08.

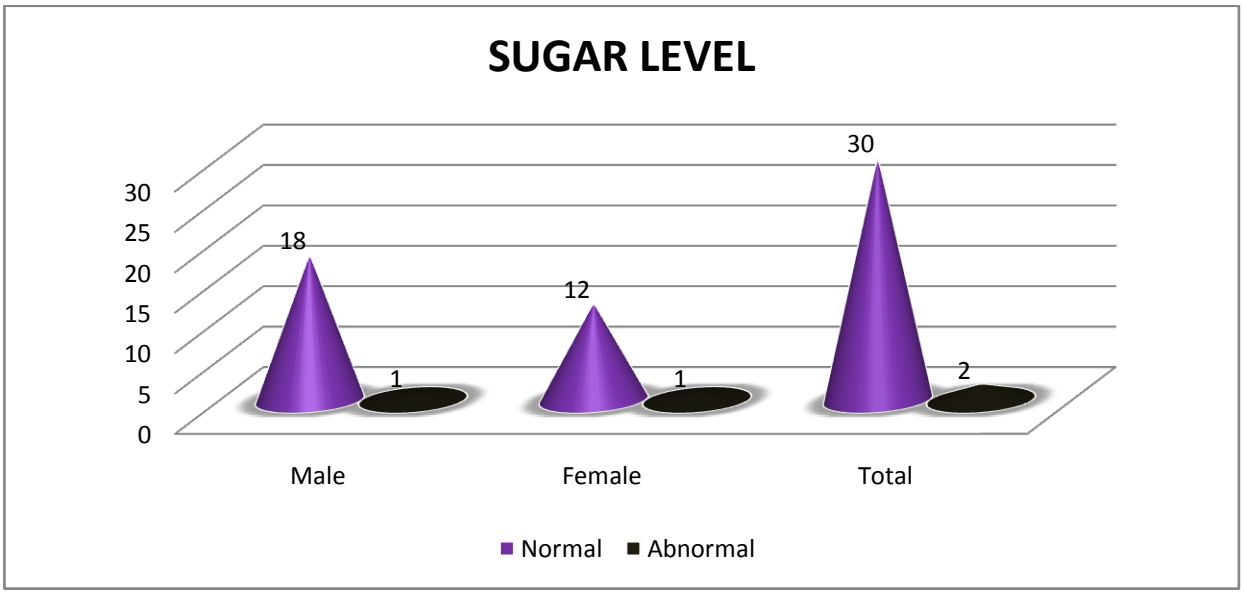
Blood urea and serum creatinine

- Normal in all 32 children
- Urea - 22.41±10.99 mg%
- Creatinine - 0.82±0.82mg%

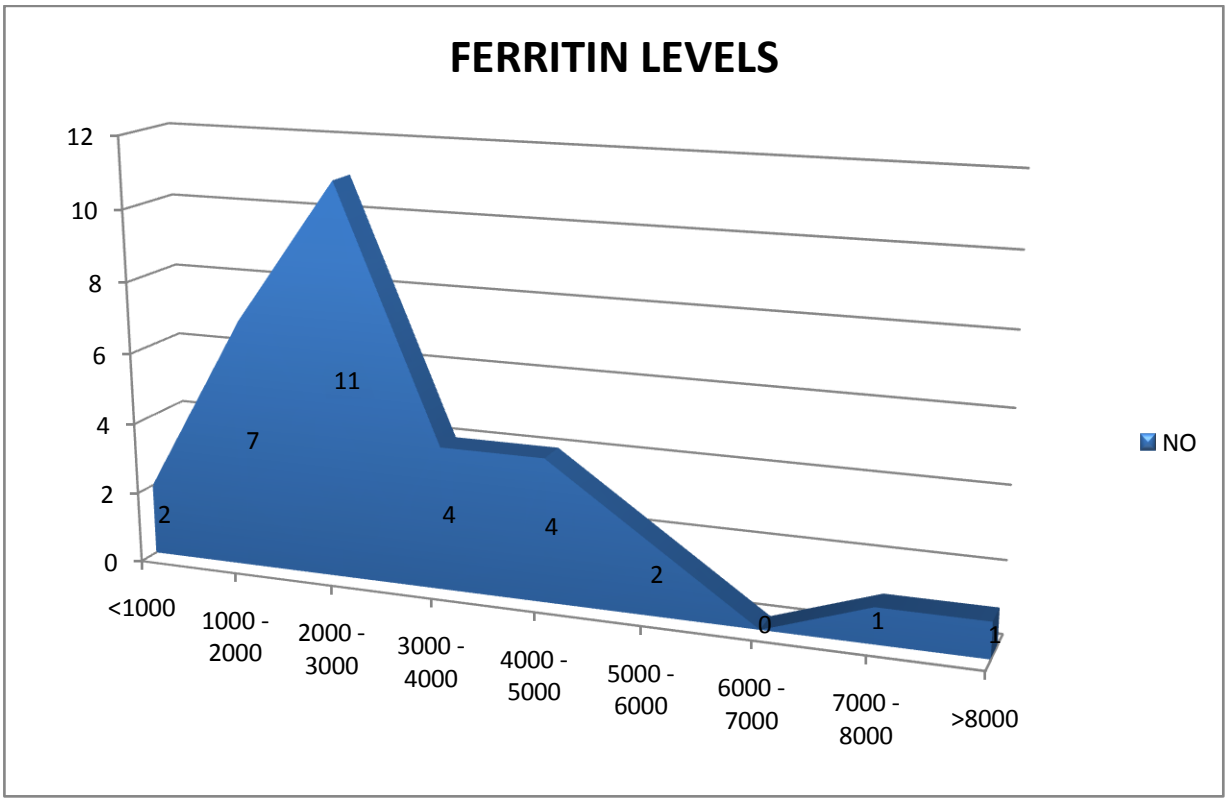
Liver function tests:

- S. bilirubin elevated in 3 out of 32 children
- Mean value: 1.47 ±1.09 mg%
- Indirect bilirubin elevated in 3 out of 32 children
- Mean value : 0.88±1.05
- SGOT elevated in 8 out of 32 children
- Mean value: 53.97±42.07 IU/L
- SGPT elevated in 19 children
- Mean value: 88.99±137.93
- Both SGPT and SGOT elevated in 8 children.
- S. total proteins decreased in 7 out of 32 children
- Mean value: 6.63 ± 0.75
- S. Albumin decreased in 19 out of 32 children
- Mean value: 3.71±0.45
- S. globulin mean value : 2.9±0.61

BLOOD SUGAR LEVEL IN STUDY GROUP



SERUM FERRITIN LEVELS IN STUDY GROUP



Endocrine abnormalities:

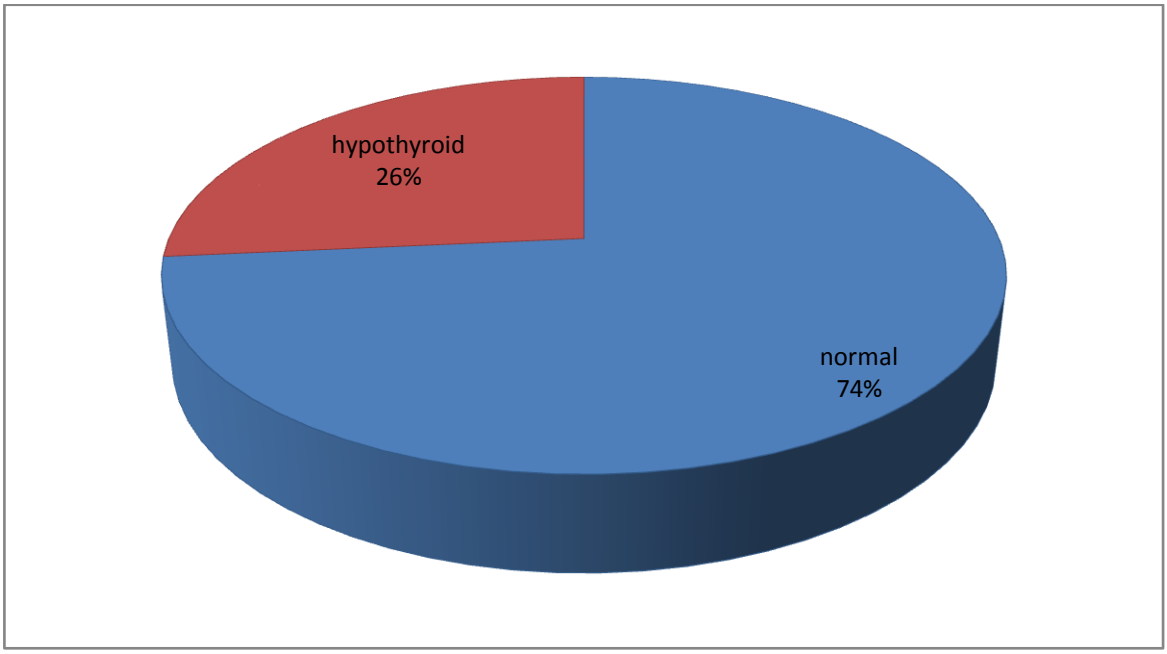
- All children were short statured
- 9 children (7 males & 2 females) had subclinical hypothyroidism (26.47%) .
- S. Calcium normal in all children

Mean values:

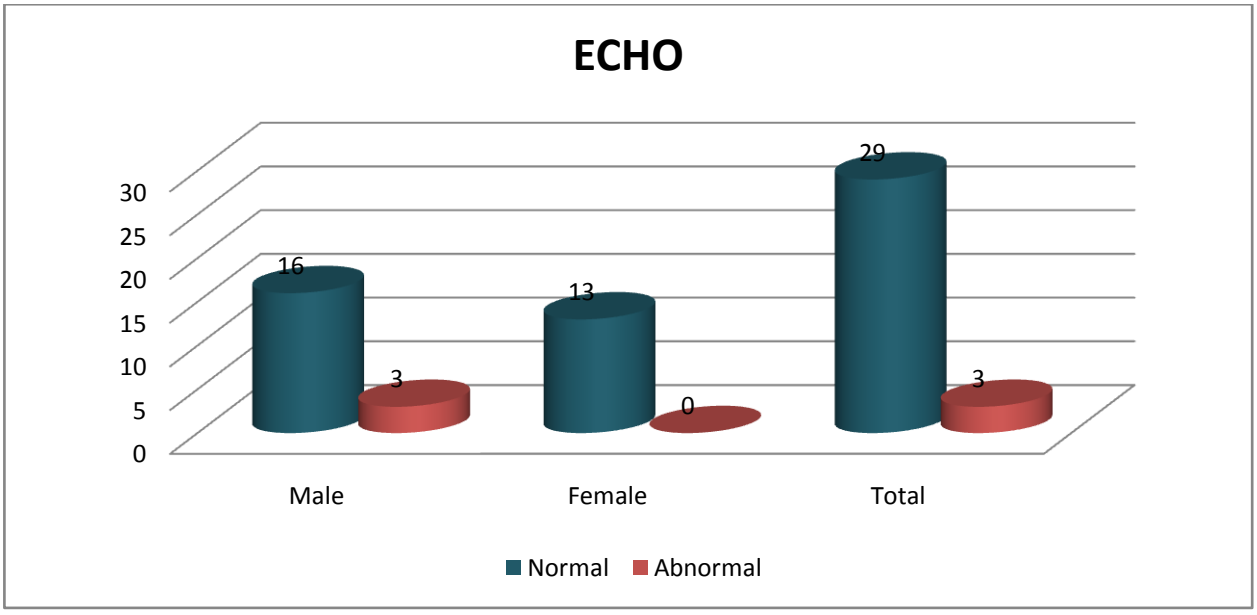
- T3 - 1.29±0.23
- T4 - 9.22±1.27
- TSH - 2.76±1.99

	1 to 6years	6 to 12 years
No of children	16	18
No. of hypothyroid	4	5
% among all children	25%	27.77%
% among hypothyroid	44.44%	55.56%
Av. Ferritin level	3138.9 ng/dl	3016.96 ng/dl

HYPOTHYROIDISM IN STUDY GROUP



CARDIAC EVALUVATION IN STUDY GROUP



Cardiac evaluation

- Normal study – 29 children
- 1 - dilated coronary sinus & small OS ASD
- 1- bicuspid aortic valve with fused raphe
- 1- slightly dilated LV with MR grade I.

Viral markers:

- 2 children among the 32 patients positive for HBsAg.

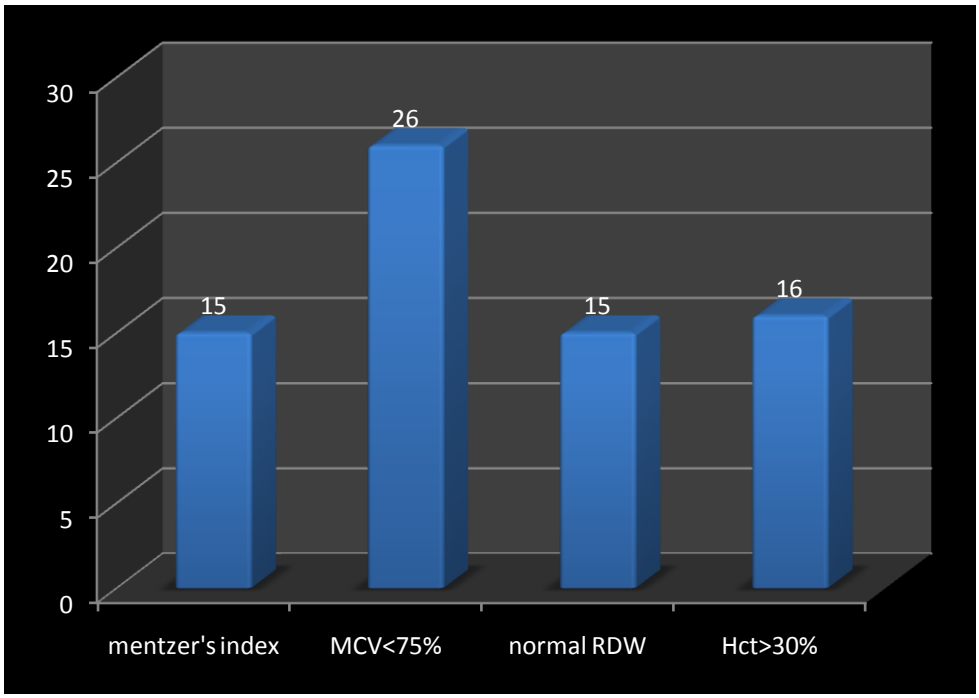
ELISA for HIV

- 2 children among the 32 patients positive for HIV

- Beta thalassemic trait was confirmed by a combination of various diagnostic parameters that include MCV <75fL, normal RDW, Hct>30%, and mentzer index <13.

All parents and 77% of siblings were found to be thalassemia traits in this study.

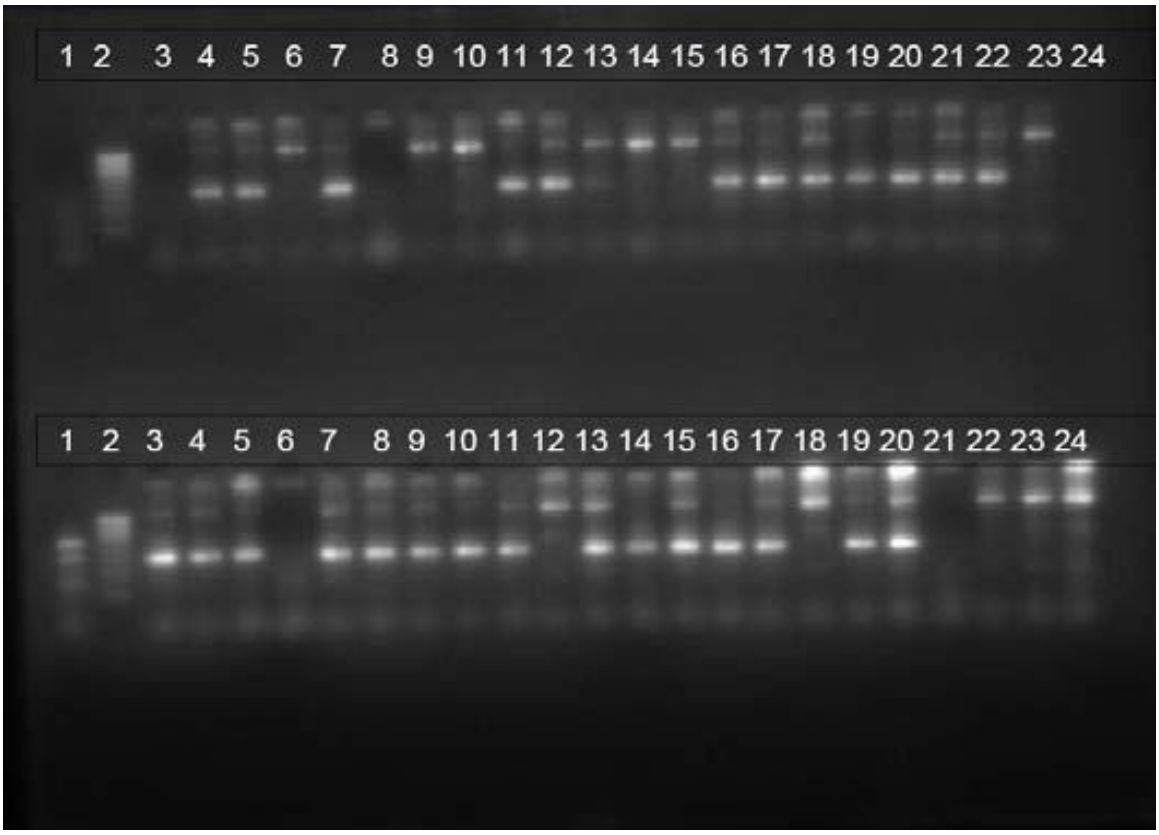
HEMATOLOGICAL PARAMETERS IN STUDY GROUP



Beta thalassemic trait was confirmed by a combination of various diagnostic parameters. Individuals with MCV <75fL, normal RDW, Hct>30%, and mentzer index <13.5 identified as carrier or thalassemic trait.

15 individuals had mentzer index <13.5, 26 individuals had MCV <75fL, 15 with normal RDW, 16 with Hct>30%. We performed molecular genotyping of beta thalassemic major mutation (IVS1-5 G to C and Cd41/42(-TCTT) in all patient and their family members.

Multiplex PCR results of the study group



PCR amplification of IVS1-5 nt (G to C) and Cd41/42 (-TCTT) mutant allele in Beta thalassemic patients

The first row lane1 is no template control, 2 DNA ladder, 3 to 23 samples

The second row: 1 positive control for mutants, 2 DNA ladder, 3 to 23 samples, 24 negative control (healthy individual).

Among the 42 samples, 16 were confirmed as thalassemia major and 25 individuals were thalassemia trait based on hematological parameters. 25 individuals (8 patients, 17 family members) out of 42 possessed IVS1-5 (G to C) and none positive for the mutant Cd41/42.

25 (8 patients, 17 individuals comprised of parents and siblings) of 42 individuals possessed the major mutant IVS1-5 (G to C) - 71% and was found to be statistically significant - $p<0.05$(Chi –Square test). None were positive for the mutant Cd41/42(-TCTT). Genetic status for the remaining samples could not be identified due to technical difficulties

RELATIONSHIP BETWEEN THYROID PROFILE AND FERRITIN

TABLE NO. 1 [A] - THYROID TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and T3	-0.107	0.56
Kendall's Tau_b	Ferritin and T3	-0.084	0.51
Spearman	Ferritin and T3	-0.134	0.46
TABLE NO. 1 [B] - THYROID TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and T4	-0.118	0.12
Kendall's Tau_b	Ferritin and T4	-0.2	0.11
Spearman	Ferritin and T4	-0.292	0.104
TABLE NO. 1 [C] - THYROID TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and TSH	0.03	0.87
Kendall's Tau_b	Ferritin and TSH	0.012	0.92
Spearman	Ferritin and	-0.03	0.87

	TSH		
TABLE NO. 1 [D] - THYROID TEST			
ANOVA	F Ratio	P Value	Significant
	101.75	O.OOO	P < 0.05

This table showed that Ferritin and T3 , Ferritin and T4 were negative correlated but TSH had positive correlation . Ferritin had significant correlation with T3,T4, TSH. It was also proved by Kendall's Tau_b and Spearman rank correlation for Ferritin and T3 and Ferritin and T4 but Ferritin and TSH showed positive correlation by Kendall's Tau_b, and negative correlation by spearman method. By analysis of variance showed significant effective changes of ferritin over T3,T4 and TSH. F ratio value was 101.750 which showed significant [P<0.05].

RELATIONSHIP BETWEEN FERRITIN AND LIVER FUNCTION TEST

TABLE NO. 2 [A] - LIVER FUNCTION TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and SGPT	0.108	0.55
Kendall's Tau_b	Ferritin and SGPT	0.135	0.28
Spearman	Ferritin and SGPT	0.181	0.32
TABLE NO. 2 [B] - LIVER FUNCTION TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and SGOT	0.273	0.13
Kendall's Tau_b	Ferritin and SGOT	0.137	0.27
Spearman	Ferritin and	0.208	0.25

	SGOT		
TABLE NO. 2 [B] - LIVER FUNCTION TEST			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and ALP	0.14	0.44
Kendall's Tau_b	Ferritin and ALP	0.158	0.2
Spearman	Ferritin and ALP	0.217	0.233
TABLE NO. 1 [D] - LIVER FUNCTION TEST			
ANOVA	F Ratio	P Value	Significant
	95.699	O.OOO	P < 0.05

This table revealed that Ferritin had positively correlated with Liver Function test. By analysis of variance showed the F Ratio was significant [p<0.05] p=0.000 .

Liver Function had significant correltion with Ferritin level. Intra class single and average measures showed positive correlation [0.367].

RELATIONSHIP BETWEEN FERRITIN AND SERUM PROTEIN

TABLE NO. 3 [A] - Protein total			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Protein	0.182	0.31
Kendall's Tau_b	Ferritin and Protein	0.029	0.82
Spearman	Ferritin and Protein	0.049	0.79
TABLE NO. 3 [D] - PROTEIN			
ANOVA	F Ratio	P Value	Significant
	101.035	O.OOO	P < 0.05

This table revealed that correlation between Ferritin and Protein was positively correlated and it is evidenced by Kendall's Tau_b and spearman method . By analysis of variance F ratio was 101.035 which was significant [$p<0.05$] Hence, Ferritin had more effective on Protein. Intra class correlation coefficient for single and average measures showed positive correlation.

RELATIONSHIP BETWEEN FERRITIN AND SERUM ALBUMIN AND GLOBULIN

TABLE NO. 4 [A] - ALBUMIN			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Albumin	-0.137	0.45
Kendall's Tau_b	Ferritin and Albumin	0.049	0.71
Spearman	Ferritin and Albumin	0.08	0.63
TABLE NO. 4 [B] - GLOBULIN			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Globulin	0.317	0.08
Kendall's Tau_b	Ferritin and Globulin	0.068	0.59

Spearman	Ferritin and Globulin	0.104	0.57
TABLE NO. 4 [C] - ALBUMIN & GLOBULIN			
ANOVA	F Ratio	P Value	Significant
	101.035	O.OOO	P < 0.05

This table showed Negative correlation between Ferritin and Albumin , Positive Correlation between Ferritin and Globulin and were significant . It is also provide by Kendall's Tau_b and spearman method by ANOVA, F ratio was 101.242 which was significant [P<0.05]

RELATIONSHIP BETWEEN FERRITIN AND BLOOD SUGAR

TABLE NO. 5 [A] - SUGAR			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Sugar	-0.062	0.73
Kendall's Tau_b	Ferritin and Sugar	-0.053	0.67
Spearman	Ferritin and Sugar	-0.076	0.679
TABLE NO. 5 [B] - SUGAR			
ANOVA	F Ratio	P Value	Significant
	94.774	O.OOO	P < 0.05

This table evidenced that correlation between Ferritin and Sugar was negatively correlated by Pearson , Kendall's tau_b and spearman methods by ANOVA ,

F ratio was 94.77 which showed P=0.000 [P<0.05] Hence, sugar had more correlation with Ferritin

RELATIONSHIP BETWEEN FERRITIN AND BLOOD UREA AND SERUM CREATININE

TABLE NO. 6 [A] - UREA			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Urea	0.139	0.45
Kendall's Tau_b	Ferritin and Urea	0.071	0.58
Spearman	Ferritin and Urea	0.102	0.58
TABLE NO. 6 [B] - CREATINE			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Creatine	0.083	0.65
Kendall's Tau_b	Ferritin and Creatine	0.123	0.35
Spearman	Ferritin and Creatine	0.169	0.36
TABLE NO. 6 [C] - UREA & CREATINE			
ANOVA	F Ratio	P Value	Significant

	100.787	O.OOO	P < 0.05

This table proved that the correlation between Ferritin and Urea, Ferritin and Creatine had positively correlated and it also proved by non para metric correlation test by ANOVA , F ratio showed significant [P<0.05] Hence, urea and creatine had more effective with ferritin.

RELATIONSHIP BETWEEN BILIRUBIN AND FERRITIN

TABLE NO. 7 [A] - SERUM BILIRUBIN			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and serum bilirubin	-0.109	0.25
Kendall's Tau_b	Ferritin and serum bilirubin	-0.138	0.29
Spearman	Ferritin and serum bilirubin	0.189	0.29
TABLE NO. 7 [B] - INDIRECT BILIRUBIN			
Name of the Test	Correlation between	Correlation Value	Significant
Pearsons Correlation	Ferritin and Indirect bilirubin	-0.172	0.35
Kendall's Tau_b	Ferritin and Indirect bilirubin	-0.081	0.54

Spearman	Ferritin and Indirect bilirubin	-0.112	0.54
TABLE NO. 7 [C] - SERUM BILIRUBIN & INDIRECT BILIRUBIN			
ANOVA	F Ratio	P Value	Significant
	101.348	O.OOO	P < 0.05

This table evidenced that correlation between SERUM BILIRUBIN and Indirect showed negative correlated. It was evidenced by Non Para Metric Test. By ANOVA ,

F ratio was 101.348 which was significant [P<0.05]

DISCUSSION

Beta thalassemia represents group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β globin chains resulting in severe anemia which needs repeated blood transfusion. The combination of transfusion and chelation therapy has dramatically improved the life expectancy of thalassemic children. On the other hand, frequent transfusion can led on to iron overload and may result in short stature , hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism, and other endocrine problems, cardiomyopathy, hepatic fibrosis and cirrhosis. In recent years, several authors have reported high incidence of these complications among patients suffering from thalassemia major.

All the 32 subjects included in the study were short statured. Historically growth retardation is generally associated with thalassemia major children and is less evident in children receiving effective chelation therapy. Alternative causes including endocrine dysfunction such as impaired growth hormone production may be considered in children who receive adequate transfusion and chelation therapy.

Incidence of short stature in other studies

Alizera A Shamshirsae et al[40]	39.3%
CK Li et al [42]	29.7%
Present study	100%

Serum ferritin levels are elevated in all children in the present study with median value of 3136.28 ±1761.44. in a study conducted in HongKong in 2002 , the mean ferritin level was found to be 5140pmolL. In general the body iron stores have been found to correlate with serum ferritin levels. However being an acute phase reactant single values of serum ferritin are not always not reliable. Despite serial measurements remains the simple and reliable method to evaluate the iron deposition and efficiency of chelation therapy. In order to evaluate clinical relevance, need for treatment, and timing and monitoring of chelation therapy, iron status should be assessed accurately.

Splenectomy has been done for 2 out of the 32 patients in the present study amounting to 6.25% compared to 37% of children who had undergone splenectomy in a study conducted in HongKong. Splenectomy should be considered if annual red cell requirement exceeds 180-200ml/kg , provided other causes if increased consumption such as infections, hemolytic reactions

have been ruled out. Symptoms of splenic enlargement, leucopenia, and/or thrombocytopenia increasing iron overload inspite of good chelation may necessitate splenectomy.

Random blood sugar estimation in the present study showed elevated levels in 2 out of 32 subjects i.e 6.25%. subsequent oral glucose tolerance tests conducted showed the presence of impaired glucose tolerance in these children.

Present study	6.25%
CK Li et al[42]	8.6%
Alizera A Shamshirsae et al[40]	8.7%
Italian working group	4.9%

In various studies the prevalence of diabetes mellitus has been reports to be between 6 -10%. Intensive iron chelation therapy is found to be associated with improvement in glucose tolerance particularly in patients with early stages of glucose intolerance.

An Indian study by Jyoti Suvarna et al concluded that diabetes mellitus or impaired glucose tolerance was not seen in chronically transfused patients and insulin resistance with compensatory hyperinsulinemia sets earlier well

before the onset of frank diabetes mellitus and correlates with the age, chelation therapy and indicators of iron overload.

Blood urea and serum creatinine is found to be normal in all subjects in the present study.

Liver is affected in due to various causes including repeated transfusions , blood borne infections, and excessive iron deposition. Elevated liver enzymes were found in 18% of thalassemic individuals in study conducted in HongKong.

Subclinical hypothyroidism is defined as normal serum T4 levels with slightly increased TSH level. In the present study, 26.47% of children (i.e 9 out of 32 children) were found to have subclinical hypothyroidism. T3 and T4 levels are found to be negatively correlating with the ferritin levels reflecting the effect of iron deposition in the thyroid gland. The prevalence of hypothyroidism is found to be between 13 -60% in patients with thalassemia major with varying severity in different series.

Present study	26.47%
Alizera A Shamshirsae et al[40]	7.7%
CK Li et al[42]	6.9%
Borgna – Pignatta et al[41]	11.6%

Treatment of subclinical hypothyroidism is debatable. Close monitoring of the patients is necessary when treatment is considered as unnecessary. In overt hypothyroidism characterized by low T4 levels with signs and symptoms such as mental and physical letharginess, cold intolerance, weight gain, constipation etc, treatment with L- thyroxine is considered. Abnormal thyroid function may be reversible t the early stage through intensive chelation therapy.

None of the children among the 32 subjects were found to have hypocalcemia. Hypoparathyroidism manifests in about 4% of thalassemic patients in various studies.

Present study	0%
Alizera A Shamshirsae et al[40]	7.6%
CK Li et al[42]	3.4%

Cardiac iron deposition have been studied in autopsies of patients with transfusional hemosiderosis. Gross anatomic cardiac changes attributable to iron overload include dilatation of atrial and ventricular cavities and overall thickening of muscle layers of heart. Moreover extent of cardiac iron deposition correlate well with the occurrence of supraventricular arrhythmias. None of the children in the present study had cardiac complications in contrast to other studies. In CK Li et al study prevalance of cardiomyopathy was 15% with the median age of onset t 16 years. Borgna – Pignatta et al study revealed the presence of heart failure in 6.4% of patients, arrhythmias in 5% of thalassemic individuals.

HbSAg was found to be positive in 2 children out of the 32 children. In CK Li et al study only 2.6% of study subjects.

HIV is positive for 2 children in our study group.

This study revealed the presence of mutation IVS1-5 G to C to be 71% and was found to be statistically significant – $p<0.05$(Chi –Square test).

An extensive study done by Colah et al in 2009 showed the prevalence of IVS1–5(G→C) mutation in Tamilnadu and Kerala to be 56.3%.Yet another study by garewal and reena das et al [46] in 2003 declared the prevalence of this

mutation to be 31.8% among Punjabis. Recent publication in 2011 showed increased prevalence of IVS1–5(G→C) in east and south India compared to northern, western and central India. A review article by Panja et al[47] in 2012 published in journal of community nutrition and health , on the contrary showed that codon 15 (G→A) to be common mutation in Tamilnadu. Major drawback in that study was that limited number of samples and studies from southern part of India compared to those obtained from northern states. But this study conducted in Madurai region, being the first of its kind among this subset of population showed that the most common prevailing mutation is IVS1–5(G→C) which is in consensus with many other studies.

CONCLUSION

- All 32 children with beta thalassemia major were on repeated packed cell transfusions at an interval of 3 to 4 weeks with an average pre transfusion hemoglobin of 5 to 7 grams.
- All 32 thalassemic patients were on irregular chelation therapy in spite of strong motivation.
- All children were short statured and malnourished indicating the underlying poor nutrition acting along with the disease pathology .
- Serum ferritin levels were invariably elevated in all patients demanding optimal chelation therapy.
- Only 3 among the 32 children were found to have cardiac ailments.
- Subclinical hypothyroidism was found in 26.45% beta thalassemic individuals which was found to be statistically significantly associated with serum ferritin levels.
- Liver functions tests showed alterations statistically significant correlation with the serum ferritin levels depicting the effect of iron overload on the liver.
- None of the children with beta thalassemia major had renal involvement.
- Serum calcium levels were normal in all thalassemic subjects

- HbSAg was positive in 2 among the 32 children
- HIV was found to positive in 2 among the 32 children
- All parents and 77% of the siblings were found to the thalassemic traits using mentzer's index
- The most common mutation among this subset of population was revealed to be IVS1–5(G→C) amounting to 71% which was found statistically significant.
- None of the study subjects were found to be positive for the mutation cd41/42.
- 77% of the siblings were thalassemic traits who are potential targets for future genetic counseling.

RECOMMENDATIONS

- Need for regular transfusion must be emphasized to improve the overall quality and duration of life of these children
- Rigid chelation therapy to prevent the dreadful complications of iron overload in thalassemic children.
- Strict follow up and monitoring for complications and prompt management should be implemented.
- Genetic counseling may be offered to siblings of these children as a measure to prevent the transmission of the disease and thereby reduce the national burden of the disease.
- Pre implantation genetics has led to new array of hope in prevention of the disease in the near future.

LIMITATIONS OF STUDY

1. Total number of patients in the study are less in number.
2. Lack of motivation among the patients and the parents is prevalent among the families.
3. Cardiac MRI, liver biopsy could not be done due to financial constraints
4. Blood samples could not be collected at the same time.
5. The major limitation of this study is some of the parents, siblings could not be sampled
6. Genetic status for few samples could not be identified yet due to technical difficulties, which will be resolved.

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PROFILE OF THALASSEMIA MAJOR CHILDREN

NAME:

ADDRESS:

HEMATOLOGY NUMBER: SERIAL NUMBER:

AGE:

SEX: MALE ☐ FEMALE ☐

RELIGION: HINDU ☐ MUSLIM ☐ CHRISTIAN ☐ OTHERS ☐

AGE AT DIAGNOSIS: <1 YR ☐ >1YR ☐

CLINICAL PRESENTATION: YES NO

ANEMIA: ☐ ☐

HEPATOMEGALY: ☐ ☐

SPLENOMEGALY: ☐ ☐

FACIAL DYSMORPHISM: ☐ ☐

JAUNDICE: ☐ ☐

FRACTURE: ☐ ☐

OTHERS: ☐ ☐

PAST HISTORY: MEASLES ☐ PC ☐ OTHERS ☐

ANTENATAL HISTORY: SIGNIFICANT ☐ NOT ☐

BIRTH HISTORY: TERM ☐ PRETERM ☐

FAMILY HISTORY:

SIBLINGS: MALE ☐ FEMALE ☐

AFFECTED ☐ NOT ☐

PARENTS: CONS. M ☐ NON CONS.M ☐

	YES	NO
H/O HEMOGLOBINOPATHY:	☐	☐
H/O OTHER ANEMIA:	☐	☐
H/O BLEEDING DISORDER:	☐	☐
H/O SPLENECTOMY:	☐	☐
H/O CHOLECYSTECTOMY:	☐	☐

IMMUNISATION HISTORY:

HEPATITIS A:	☐	☐
HEPATITIS B:	☐	☐

INVESTIGATIONS:

BL. GROUPING & TYPING: A ☐ B ☐ AB ☐ O ☐

POSITIVE ☐ NEGATIVE ☐

BL. HEMOGLOBIN: <5g% ☐ 5-7g% ☐ 7-11g% ☐ >11g% ☐

COOMB'S TEST: POSITIVE ☐ NEGATIVE ☐

RETICULOCYTE COUNT: ☐

RDW: ☐

PERIPHERAL SMEAR: ☐

LDH: ☐

HB ELECTROPHORESIS: ☐ ☐ ☐

S. IRON:

S.FERRITIN:

TIBC:

BL. SUGAR:

GTT : 0HRS ½ HR 1HR 2HRS

BL.UREA:

S.CREATININE:

S.BILIRUBIN- TOTAL: - DIRECT: INDIRECT:

SGOT:

SGPT:

ALP:

S. CALCIUM:

S.TSH:

S.T3:

S.T4:

VIRAL MARKERS:	POSITIVE	NEGATIVE
HbSAg:		
HEPATITIS A:		
HEPATITIS C:		
ELISA FOR HIV:		

IMAGING:

XRAY SKULL

HAIR ON END APPEARANCE

XRAY CHEST

CARDIOMEGALY ☐ ☐

ECG:

ECHO:

TREATMENT DETAILS

MODE OF TREATMENT: YES NO

PACKED CELL TRANSFUSION: ☐ ☐

SPLENECTOMY: ☐ ☐

HYDROXY UREA/ OTHERS: ☐ ☐

TRANSFUSION DETAILS

TOTAL NO. OF TRANSFUSION:

INTERVAL BETWEEN

EACH TRANSFUSION: 3WKS 4WKS 6WKS

☐ ☐ ☐

CHELATION DETAILS:

REGULAR CHELATION: ☐

IRREGULAR CHELATION: ☐

NO CHELATION: ☐

PHLEBOTOMY DONE: YES NO

☐ ☐

Ref. No. 14290 /E4/3/2012

Govt. Rajaji Hospital,
Madurai.20. Dated: . 12.2012

Institutional Review Board / Independent Ethics Committee.
Dr. N. Mohan, M.S., F.I.C.S., F.A.I.S.,
Dean, Madurai Medical College &
Govt Rajaji Hospital, Madurai- 625020.
Convenor
grhethicssecy @gmail.com.

Sub: Establishment-Govt. Rajaji Hospital, Madurai-20-
Ethics committee Meeting- approval -regarding.

The Ethics Committee meeting of the Govt. Rajaji Hospital, Madurai was held at 10.00 am to 12.30.Pm on 10.12.2012 at the Surgery Seminar Hall, Govt. Rajaji Hospital, Madurai. The following members of the committee have attended the meeting.

1.Dr. V. Nagarajan, M.D., D.M (Neuro) Ph: 0452-2629629 Cell.No 9843052029	Professor of Neurology (Retired) D.No.72, Vakkil New Street, Simmakkal, Madurai -1	Chairman
2. Dr.Mohan Prasad , M.S M.Ch Cell.No 9843050822 (Oncology)	Professor & H.O.D of Medical Oncology(Retired) D.No.72, West Avani Moola Street, Madurai -1	Member Secretary
3. Dr.L. Santhana Lakshmi,MD Cell.No 9842593412	Associate Professor of Physiology/V.P Madurai Medical College	Member
4. Dr. Parameswari M.D (Pharmacology) Cell.No.9994026056	Director of Pharmacology Madurai Medical College	Member
5. Dr.Moses K.Daniel MD(Gen.Medicine) Cell.No 09842156066	Professor & H.O.D of Medicine Madurai Medical College	Member
6. Dr.D. Soundara Rajan,MS(Gen.Surgery) Cell.No 9842120127	Professor & H.O.D of Surgery Madurai Medical College	Member
7. Dr.Angayarkanni MD(O&G) Cell.No 9443567724	Professor & H.O.D of O&G Madurai Medical College	Member
8. Dr.P.V. Pugalenthi M.S, (Ortho) Cell.No 9443725840	Professor & H.O.D Ortho Madurai Medical College	Member
9. Dr. M. Sundarajan M.S., Mch Cell.No 9994924369 (Neuro Surgery)	Professor (Neuro Surgery) Madurai Medical College	Member
10 Thiru..Pala. .Ramasamy . BA.,B.L., Cell.No 9842165127	Advocate, D.No.72.Palam Station Road, Sellur, Madurai -2	Member
11. Thiru. P.K.M. Chelliah ,B.A Cell.No 9894349599	Businessman, 21 Jawahar Street, Gandhi Nagar, Madurai-20.	Member

The following Project was approved by the committee

Name of P.G.	Course	Name of the Project	Remarks
Dr. M.S Nisha	PG in M.D., Pediatrics Madurai Medical College, Madurai -20.	A Study on Clinical Profile of Beta Thalassemia major children.	Approved

Please note that the investigator should adhere the following: She/He should get a detailed informed consent from the patients/participants and maintain Confidentially.

1. She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the institution to Government.
2. She/He should inform the institution Ethical Committee in case of any change of study procedure site and investigation or guide.
3. She/He should not deviate for the area of the work for which applied for Ethical clearance. She/He should inform the IEC immediately, in case of any adverse events pr Serious adverse reactions.
4. She/he should abide to the rules and regulations of the institution.
5. She/He should complete the work within the specific period and apply for if any Extension of time is required She should apply for permission again and do the work.
6. She/He should submit the summary of the work to the Ethical Committee on Completion of the work.
7. She/He should not claim any funds from the institution while doing the word or on completion.
8. She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

Member Secretary **Chairman**

DEAN/Convenor
Govt. Rajaji Hospital,
Madurai- 20.

6/4/12 Adhoc (1/7)

To
The above PG student - thro' Head of the Department concerned.

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by Nisha 20103103 M.D.

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DY ON CLINICAL PROFILE OF BETA THALASSEMIA MAJOR CHILDREN DISSERTATION MITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF DOCTOR OF MEDICINE BRANCH (PAEDIATRICS) APRIL – 2013 THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY CHENNAI MILNADU CERTIFICATE This is to certify that this dissertation titled study on dinical profile of beta assemia major children submitted by DR.M.S.NISHA to the Tamilnadu DR. M.G.R medical versity, Chennai in partial fulfilment of the requirement for the award of MD degree branch VII, is a nafide research work carried out by her under direct supervision and guidance. DR.CHITRA YAPPAN DR.G.MATHEVAN Professor of paediatrics, Director i/c, Madurai...

MASTER CHART																					
S.No	Name	Age	Sex	Sugar	Urea	Creatinine	SBR	Indirect	SGPT	SGOT	ALP	Protein	Alb	Globulin	Echo	Ferritin	T3	T4	TSH	Cons	District
1	alageshwari	2	f	90	18	0.6	3.5	3	37	19	130	7.8	4.7	3.1	normal study	2611.6	1.13	7.5	1.24	2nd	ramnad
2	alex pandian	7	m	86	15	0.5	1.5	1	35	35	130	6.8	4	2.8	normal study	1632.9	1.64	11.6	2.71	ncm	tirunelveli
3	arun	5	m	121	19	0.6	0.9	0.3	29	28	71	5.2	3.1	2.1	normal study	1797	1.49	11.3	2.08	3rd	viruthungr
4	azhahar	10	m	83	19	0.8	1	0.4	63	73	263	7.1	3.6	3.5	normal study	2546	1.09	10.3	6.96	3rd	mdu
5	deepak kumar	3	m	96	26	0.7	4.5	4	29	38	122	6	4	2	normal study	1994	1.06	7.6	2.41	3rd	mdu
6	deepan	5	m	94	31	0.8	1	0.6	100	86	354	5	3	2	normal study	854	1.2	9.2	2.36	ncm	dindugal
7	dharanishri	5	f	84	22	0.8	0.8	0.4	44	44	123	6.8	4	2.8	normal study	2654	1.3	8.6	1.92	3rd	mdu
8	dharmar	11	m	81	20	0.6	0.9	0.5	115	80	227	7.7	3.2	4.5	normal study	8792	1.19	8.2	1.86	2nd	mdu
9	hari	5	m	130	16	1	0.7	0.2	65	25	165	6.3	3.3	3	normal study	5246.8	1.43	8.3	2.85	ncm	theni
10	jagadeshwari	8	f	88	22	0.8	1	0.5	29	37	130	6.1	4	2.1	normal study	3870.5	0.83	10.2	8.65	ncm	virudhungr
11	karthik	9	m	132	18	1.1	1.2	0.4	56	40	45	7	3.7	3.3	dilated coronary sinus; small ASD os	2758.8	0.96	10	0.92	3rd	mdu
12	maheshraj	5	m	102	20	0.9	1	0.4	60	42	90	6.4	3.8	2.6	normal study	2880.8	1.4	7.8	1.4	3rd	mdu
13	mathimalar	5	f	100	27	0.9	1.5	1	292	167	282	7	3.4	3.6	normal study	7135.3	1.2	7.2	2.38	3rd	theni
14	mohamed arif	2	m	90	22	0.7	1	0.2	0.8	32	100	7.1	3.9	3.2	normal study	943	1.2	8	1.4	3rd	mdu
15	mohana	1	f	60	16	0.5	1.2	0.4	392	224	635	7	4.2	2.8	normal study	3200	1.4	9	1.32	3rd	mdu
16	muthupandi	8	m	84	26	0.6	0.9	0.4	54	46	243	5.6	3.2	2.4	normal study	5921.7	1.6	10.4	1.2	3rd	mdu
17	naveen kumar	3	m	72	29	1.3	1	0.5	48	18	147	5.8	3.2	2.6	normal study	2228.8	1.39	11.5	3.65	ncm	mdu
18	nebura	6	f	170	14	1.1	1	0.4	62	44	38	7.4	4	3.4	normal study	1209	1.4	9.8	2.4	3rd	mdu
19	pandimeena	5	f	81	19	0.8	0.9	0.4	77	88	190	7.4	3.4	4	normal study	1752.7	1.15	9.8	3.01	2nd	mdu
20	praveen	8	m	294	25	1.1	1	0.4	67	80	92	7	3.5	3.5	bicuspid aortic valve with fused raphe, AR mild	3205	1.2	8.4	2.32	3rd	mdu
21	rajalakshmi	11	f	69	78	2	2	1.5	40	39	251	6.9	4	2.6	normal study	4338	1.02	9.9	4.07	ncm	madurai
22	rajeshwari	12	f	84	20	0.6	1	0.5	48	38	112	6.8	4	2.6	normal study	2904	1.15	7.8	2.1	3rd	mdu
23	ramana	9	m	80	24	0.8	1.2	0.4	48	41	103	7	4	3	normal study	4656.7	1.3	8.2	1.92	3rd	mdu
24	ramya	9	f	87	23	0.9	1	0.5	47	44	111	5	3	2	normal study	2188.9	1.49	8.4	2.51	2nd	virudhungr
25	riyaz	11	m	103	16	0.9	0.7	0.3	38	37	75	6.6	4.3	2.3	normal study	4501.4	0.9	9	6.16	3rd	mdu
26	rohini	9	f	83	16	0.7	1	0.4	24	57	159	6.4	3.4	3	normal study	4068	1.46	9.1	1.86	ncm	dindugal
27	sabarish	2 1/2	m	102	23	0.9	0.9	0.4	62	45	238	5.9	3.4	2.5	normal study	2580.9	1.99	10.9	5.27	3rd	theni
28	saravanakumar	5	m	92	20	0.8	1.1	0.5	44	41	102	6.9	4	2.9	normal study	3421	1.4	9.2	1.4	3rd	theni
29	selvaraj	9	m	80	20	0.6	5	4	40	35	48	6.4	3.8	2.6	normal study	1640.5	1.37	9.9	0.35	3rd	mdu
30	subashri	4	f	90	18	0.6	3.5	3	37	19	130	7.8	4.7	3.1	normal study	2611.6	1.13	7.5	1.24	2nd	ramnad
31	yogashri	5	f	100	14	0.6	2	1	720	50	580	7	3.2	3.8	normal study	2324.2	1.37	11.1	7.23	3rd	mdu
32	yuvan raja	3	m	84	21	0.6	1.2	0.4	45	35	124	7	3.8	3.2	slightly dilated LV, MR grade 1	1892	1.3	9.2	1.3	3rd	theni